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Original Articles

PREVALENCE OF FIVE BRCA1/2 MUTATIONS IN BULGARIAN BREAST CANCER PATIENTS

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Summary

Detection of mutations in breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) gene is an effective method of early diagnosis and prevention of breast cancer (BC). The mutational spectrum of both genes in Bulgarian population has not been studied in depth. The aim of this study was to investigate the prevalence of five deleterious BRCA1/2 point mutations in high-risk BC women, selected according to the National Comprehensive Cancer Network (NCCN) Guidelines including early age of onset, triple-negative BC and family history of breast or ovarian cancer. The prevalence of two BRCA1 mutations (C61G and 5382insC) and three BRCA2 mutations (6079del4, 9326insA and 9908delA) was evaluated in 80 females with BC, obtained from the Cancer Registry of University Hospital – Pleven. Genetic testing was performed by direct DNA sequencing. One deleterious mutation (5382insC in exon20 in BRCA1) was been found in two patients (2.5%). Both women were diagnosed with BC before age 45. The prevalence of BRCA mutations established in our study was lower than the one found in another preliminary study on Bulgarian population. We concluded that this discrepancy was due to the genetic heterogeneity of the population and the specific mutational spectrum of the BC patients from the Pleven region.
Key words: BRCA1, BRCA2, breast cancer (BC), Bulgarian population, mutation

Introduction

Breast cancer (BC) is the most common cancer and a leading cause of cancer death in women worldwide. In Bulgaria, one of 14 women will develop BC, during her lifetime and the cases of BC have increased continuously during the last years [1]. BC is a multifactorial disorder, caused by the interaction of genetic alterations in susceptibility genes and environmental factors. The main BC susceptibility genes are breast cancer 1 gene (BRCA1) and breast cancer 2 gene (BRCA2), localized on the long arm of chromosome 17 and chromosome 13, respectively. The germline mutations in these genes play a major role in the etiology of hereditary BC (about 5% of all BC cases) [2]. Healthy carriers of deleterious mutation in BRCA1/2 have a high (up to 80%) risk to develop BC during their life. BRCA-linked BC have some specific features such as early age of onset,

bilaterality, familial segregation of breast and/or ovarian cancer, as well as some histopathological characteristics such as triple-negative status of estrogen, progesterone and human epidermal growth factor receptor 2 (HER2) receptors. The detection of BRCA mutation in healthy women allows taking some prophylactic measures such as intensive diagnostic monitoring and prophylactic surgery in some cases.

In the Breast Cancer Information Core (BIC) database, 1787 distinct germline mutations in BRCA1gene, and 2000 in BRCA2 gene have been recorded. About 54% (969) of these mutations and 53% (1065), respectively, have been reported just once [2]. The mutations appear to be equally distributed across the coding sequences, with no obvious "hot-spot mutations" [3, 4]. Comprehensive testing of BRCA mutation requires sequencing of entire coding regions of both BRCA1 and BRCA2 genes. This technique is expensive, even for wealthy countries, so in practice the screening of the two genes should be initiated with searching for the most common genetic alterations, specific for the given population. In genetically homogeneous populations such as Ashkenazi Jews (AJ), there are the three most common mutations called "founder" (185delAG and 5382insC in BRCA1, and 6174delT in BRCA2) and they cover nearly all BRCA defects. In Iceland, BRCA2 999del5 is the most common defect in cases of hereditary BC. In these and some other ethnic groups, the detection of BRCA1/2 mutations by non-expensive molecular-genetic techniques appears to be a reasonable and cost-efficient diagnostic approach [5-7].

The genetic heterogeneity of a population such as the Bulgarian, as well as the insufficient knowledge about the spectrum of specific BRCA1/2 mutations makes the development of a strategy for genetic testing in BC patients very complicated.

Given these facts, we tried to investigate the prevalence of five deleterious BRCA1/2 mutations in Bulgarian BC women from the Pleven region.

Materials and Methods

Patients

The list of the patients diagnosed with BC and the information regarding their clinical history were obtained from the Cancer Registry of the University Hospital – Pleven for 2009, 2011 and 2012.

Letters with information about the aim of the study and invitations to participate were sent to all live female patients selected from the Cancer Registry. The study was approved by the Ethics and research committees of the hospital.

The patients who responded to the invitation visited the Section of Medical Genetics at the Medical University, Pleven. A questionnaire prepared in advance was filled by a genetic counselor during the interview with each woman. The questionnaire included the following information: 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, physical activity, 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. The patient with BC was defined as proband (individual from whom the family history was traced).

The probands were classified into ten categories, according to their family and personal disease history: (1) early-onset BC (<40 years); (2) Triple negative BC (TNBC); (3) familial BC – the occurrence of two or more first degree relatives in the pedigree including the proband; (4) bilateral BC; (5) early-onset BC and TNBC; (6) early-onset and family history of BC; (7) no family history but with both BC and ovarian cancer (OC); (8) TNBC and family history; (9) age of onset of BC between 40 and 50 years; (10) without any of the criteria mentioned above.

Based on the National Comprehensive Cancer Network (NCCN) Guidelines, we defined criteria for selecting patients to be screened for the BRCA mutations. The target group included all women from the first nine groups. They were referred for genetic testing after signing an informed consent form.

Based on the results of a previous study on a Bulgarian population [8] and using the BIC database [2] we selected five specific mutations for genetic testing (c.181T>C / C61G/ and c.5263_5264insC /5382insC/ in BRCA1 and c.5851_5854delAGTT /6079del4/,

c.9098_9099delA /9326insA/, and c.9680delA /9908delA/ in BRCA2). The molecular sequencing analysis was performed at the Molecular Medicine Center, Medical University – Sofia.

Sequencing of BRCA1 and BRCA2 genes for the selected mutation

Genomic Deoxyribonucleic acid (DNA) was isolated from peripheral blood using standard protocols (CHEMAGEN® Magnetic Separation Station). To amplify exons 2 and 20 of BRCA1 and exons 11n and 17 of BRCA2 and exons boundaries, primer pairs were used. Primer selection was made from BIC database [9].

Genomic DNA was amplified by the Polimerase Chain Reaction (PCR). The 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 Human Genome Variation Society (HGVS – nomenclature guidelines for cDNA sequence) and The Single Nucleotide Polymorphism Database (dbSNP).

Results

A total of 662 females with BC were recorded in the Cancer Registry of University Hospital, Pleven for the study period. Forty-two of them were dead. An invitation to take part in our study was sent to the rest 620 live females and 170 (27.0%) of them agreed to participate. Six more patients were referred from the Department of Surgical Oncology, University Hospital of Pleven. The final total number of patients was 176. The average age of the patients at time of diagnosis was 57 (range 24-69) years. They were interviewed and pedigrees were constructed.

The main characteristics of all the 176 women interviewed, according to the selection criteria, are presented in details on Table 1. A total of 20 (11.4%) women demonstrated familial BC, 21(11.9%) – early onset BC, 20 (11.4%) – had TNBC, and only four women (2.3%) had bilateral BC.

Finally, 80 women met the selection criteria of target group and were screened for the mutations. The average age at diagnosis of the tested women was 50 years. We detected one (5382insC in exon 20 of BRCA1 gene) of the screened mutations in two patients (2.5%). One of the women was diagnosed with TNBC at age 39. The other one had been diagnosed with BC at age 43, and her mother had been diagnosed with ovarian cancer at the age of 56. The other four selected mutations were not detected among the studied patients.

Table 1. Characteristics of the interviewed patients

Characteristics	Number of patients (n=176)	%
Early age of onset (<40)	14	8
Triple-negative BC (TNBC)	16	9
Familial BC	17	10
Bilateral BC	4	2
Early age of onset (<40)+TNBC	5	3
Early age of onset (<40)+Familial BC	2	1.8
BC and Ovarian cancer	1	0.6
TNBC+Familial BC	1	0.6
BC with age at diagnose 40-50 years	20	11
Without any specific features	96	55

Discussion

Genetic testing for mutations in BRCA1 and BRCA2 has become a routine part of the investigation and management of BC worldwide. In this study, a total of 80 Bulgarian BC female patients were tested for five BRCA mutations and one of these mutations (5382insC in BRCA1) was detected in two of the women. We identified a low prevalence (2.5%) of deleterious mutation in BRCA1/2, compared to another previous study [8] for Bulgarian population with established prevalence of 14%. A similar low prevalence (0-7% for BRCA1; 1-3% for BRCA2) was reported for other European populations in a review article [10].

What is the possible explanation of the

relatively low frequency determined in our study? Partially, it is due to the strong used criteria (family history of cancer, early age of onset or TNBC) that we borrowed from studies on other European populations. Another possible factor is the selection of a limited number of tested mutations, considered as the most frequent in the BIC database. In actual fact, the approach of using strong preliminary selection criteria is more efficient mainly in genetically homogeneous populations with well-known BRCA genetic defects and might be not appropriate for the Bulgarian population. In addition, the studies [11-13] in other countries such as Poland, Norway, Belgium have shown that a significant proportion of BRCA mutation carriers actually have not met these strong criteria. Furthermore, the biotech company Myriad Genetics has recently suggested that the criteria for genetic testing of BRCA1/2 be broadened to give a better opportunity for identification of mutation carriers [14].

Many studies indicate that genetic testing is more efficient and less cost-consuming when there is preliminary information about the founder mutations in a particular population. Concerning the Bulgarian population, there is still insufficient knowledge about the most common genetic defects and the choice to test only for five specific genetic defects could reduce the opportunity to detect the mutation carriers at all.

The only detected mutation in our study was 5382insC, found in two of the tested BC patients. This mutation was also observed in a wide range for some other European populations. A recent study has shown that the mutation is not merely an AJ founder mutation but in fact appears to be the most common BRCA1 mutation in several European countries. The defect originated in Northern Europe, especially Russia or possibly Denmark, between 1800 and 1500 years ago, and after a sequence of events slowly spread west and south to the rest of Europe from the Russian plains with the Slavic migration. So we could call 5382insC a "Slavic" "mutation [15]. The mutation 5382insC was found in high-risk breast and/or ovarian cancer families and reported with a frequency variation for different populations and countries: Poland (34%) [7], Russia (14%) [16], Hungary (14%) [17], Slovenia (13%) [18], AJ (10%) [19], Greece (8%) [20], Germany (4%) [21], Italy (3%) [22]. Furthermore, this genetic defect is virtually absent in Spain and Portugal and it is found at extremely low frequency in the Netherlands, Belgium and Scandinavian countries [23].

A preliminary study on a Bulgarian population showed a higher (11.5%) frequency of 5382insC, as compared with this study. Probably, the discrepancy is due to the genetic heterogeneity of our population and the specific mutational spectrum of the BC patients from the Pleven region.

Conclusions

Despite of the advances in the treatment of BC, the prophylactic measurements are the only reliable way to manage this disorder. Genetic testing for BRCA mutation is a well-recognized management tool for BC prevention in many countries. The high cost of BRCA testing is still not available for Bulgarian patients, because of the genetic heterogeneity of the population and the large number of reported genetic alterations in BRCA1 and BRCA2 genes.

The genetic testing for specific mutations was performed on a small group of tested patients, without basic information concerning the founder mutations, and appears to be ineffective. In order to increase the rate of mutation detection, the whole genes sequencing is recommendable for future investigations.

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References

1. Bulgarian national cancer registry. [Cancer incidence in Bulgaria – data for 2010]. [Internet] 2018 [cited 2018 Nov 27]. Available from: http://www.sbaloncology.bg/en/ncr.html. Bulgarian.
2. National Human Genome Research Institute. Breast cancer information core [Internet] 2017 [cited 2018 Oct 14]. Available from: http://research.nhgri.nih.gov/projects/bic/
3. Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, et al. Prevalence of BRCA1

and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst. 1999;91(11):943-9.
4. Serova OM, Mazoyer S, Puget N, Dubois V, Tonin P, Shugart YY, et al. Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? Am J Hum Genet. 1997;60(3):486-95.
5. Neuhausen SL. Founder populations and their uses for breast cancer genetics. Breast Cancer Res. 2000;2(2):77-81.
6. Phelan CM, Kwan E, Jack E, Li S, Morgan C, Aubé J, et al. A low frequency of non-founder BRCA1 mutations in Ashkenazi Jewish breast-ovarian cancer families. Hum Mutat. 2002;20(5):352-7.
7. Górski B, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Grzybowska E, et al. A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer 2004;110(5):683-6.
8. Dodova RI, Mitkova AV, Dacheva DR, Hadjo LB, Vlahova AI, Hadjieva MST, et al. Spectrum and frequencies of BRCA1/2 mutations in Bulgarian high risk breast cancer patients. BMC Cancer. 2015 Jul.
9. National Human Genome Research Institute. Breast cancer information core [Internet] 2017. [cited 2018 Oct 14]. Available from: http://research.nhgri.nih.gov/projects/bic/Member/brca1_mutation_database.shtml.
10. Fackenthal JD, Olopade OI: Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat Rev Cancer. 2007;7(12):937-48.
11. Gaj P, Kluska A, Nowakowska D, Bałabas A, Piatkowska M, Dabrowska M, et al. High frequency of BRCA1 founder mutations in Polish women with nonfamilial breast cancer. Fam Cancer. 2012;11(4):623-8.
12. Møller P, Hagen AI, Apold J, Maehle L, Clark N, Fiane B, et al. Genetic epidemiology of BRCA mutations – family history detects less than 50% of the mutation carriers. Eur J Cancer. 2007;43(11):1713-7.
13. De Leeneer K, Coene I, Crombez B, Simkens J, Van den Broecke R, Bols A, et al. Prevalence of BRCA1/2 mutations in sporadic breast/ovarian cancer patients and identification of a novel de novo BRCA1 mutation in a patient diagnosed with late onset breast and ovarian cancer: implications for genetic testing. Breast Cancer Res Treat. 2012;132(1):87-95.
14. Hartman AR, Kaldate RR, Sailer LM, Painter L, Grier CE, Endsley RR, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. Cancer. 2012;118(11):2787-95.
15. Hamel N, Feng BJ, Foretova L, Stoppa-Lyonnet D, Narod SA, Imyanitov E et al. On the origin and diffusion of BRCA1 c.5266dupC (5382insC) in European populations. Eur J Hum Genet. 2011;19(3):300-6.
16. Sokolenko AP, Mitiushkina NV, Buslov KG, Bit-Sava EM, Iyevleva AG, Chekmariova EV, et al. High frequency of BRCA1 5382insC mutation in Russian breast cancer patients. Eur J Cancer. 2006;42(10):1380-4.
17. Ramus SJ, Kote-Jarai Z, Friedman LS, van der Looij M, Gayther SA, Csokay B, et al. Analysis of BRCA1 and BRCA2 mutations in Hungarian families with breast or breast-ovarian cancer. Am J Hum Genet. 1997;60(5):1242-6.
18. Krajc M, Teugels E, Zgajnar J, Goelen G, Besic N, Novakovic S, et al. Five recurrent BRCA1/2 mutations are responsible for cancer predisposition in the majority of Slovenian breast cancer families. BMC Med Genet. 2008;9:83.
19. Couch FJ, Weber BL. Mutations and polymorphisms in the familial early-onset breast cancer (BRCA1) gene. Breast Cancer Information Core. Hum Mutat. 1996;8(1):8-18.
20. Ladopoulou A, Kroupis C, Konstantopoulou I, Ioannidou-Mouzaka L, Schofield AC, Pantazidis A, et al. Germ line BRCA1 & BRCA2 mutations in Greek breast/ovarian cancer families: 5382insC is the most frequent mutation observed. Cancer Lett. 2002;185(1):61-70.
21. Backe J, Hofferbert S, Skawran B, Dörk T, Stuhrmann M, Karstens JH, et al. Frequency of BRCA1 mutation 5382insC in German breast cancer patients. Gynecol Oncol. 1999;72(3):402-6.
22. Caligo MA, Ghimenti C, Cipollini G, Ricci S, Brunetti I, Marchetti V, et al. BRCA1 germline mutational spectrum in Italian families from Tuscany: a high frequency of novel mutations. Oncogene. 1996;13(7):1483-8.
23. Ferla R, Calò V, Cascio S, Rinaldi G, Badalamenti G, Carreca I et al. Founder mutations in BRCA1 and BRCA2 genes. Ann Oncol. 2007;18 Suppl 6:vi93-8.