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Original Article

Stepwise approach to hereditary breast cancer and evaluation of BRCA negative patients

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Abstract

Aim: Hereditary breast cancer is one of the components of hereditary breast-over cancer syndrome (HBOC) that results with multiple cancer predisposition. Heterozygous mutations of *BRCA1* and *BRCA2* that have crucial roles in DNA repair are responsible for about 12-25% of the patients. 25 different genes have been identified to be a causal factor in the patients whose BRCA tests are negative. Most of these genes are involved in functionally related genome maintenance pathways with *BRCA1* and *BRCA2*. We aimed to investigate genes other than *BRCA1*/2 to determine breast cancer etiology.

Materials and Methods: Of the 230 patients diagnosed with breast cancer, 38 patients with no mutation in *BRCA1* / 2 were evaluated. Hereditary breast cancer panel was designed with QIAseq solution. The sequencing process was performed on the Illumina MiSeq system. The data analyses were performed on QIAGEN Clinical Insight analyze software.

Results: The genes associated with hereditary breast cancer were studied with the new generation sequencing method in 38 of the patients without pathogenic or VUS variants. Pathogenic/likely pathogenic variants were detected in 3 (7.8%) of 38 patients, while VUS was detected in 10 (26.3%) patients. Of these 22 genes, c.312C>A (p.Tyr90Ter) in MUTY, c.1225C>T (p.Arg409Trp) in *STK11*, c.1690C>T (p.Gln564Ter) variant in *BARD1* interpreted as pathogenic.

Conclusion: Our data provide insight into the genetics of HBOC syndrome in Turkey. These studies will help to improve the clinical management and better risk assessment of hereditary breast and ovarian cancer.

Keywords: Hereditary breast-over cancer syndrome; hereditary cancer panel; next generation sequencing

INTRODUCTION

Breast cancer ranks first among female cancers in terms of incidence and mortality, worldwide (1). Approximately 10% of all breast cancers are thought to be hereditary. Additionally, about 20% of the patients with an extra affected individual in the family have a germline pathogenic mutation of a hereditary cancer syndrome gene (2, 3). Hereditary breast cancer is one of the components of hereditary breast-over cancer syndrome (HBOC) that results with multiple cancer predisposition. Heterozygous mutations of the two tumor suppressor genes *BRCA1* and *BRCA2* that have crucial roles in DNA repair are responsible for about 12-25% of the patients with HBOC (4). HBOC has a great locus heterogeneity that nearly 25 different genes have been identified to be a causal factor in the patients whose BRCA tests are negative. Most of these genes are involved in functionally related genome maintenance pathways with *BRCA1* and *BRCA2* (5).

Several breast cancer screening programs are used to be applied in various populations, due to the risk factors including demographic, reproductive, hormonal, and lifestyle factors as well as hereditary susceptibility (6). Socio-economic status and literacy of health in a population are the other important factors for early diagnosis and prevention of breast cancer, consequently the mortality rate is higher in underdeveloped and developing countries (7). With the spread of cancer risk assessments and screening programs, early diagnosis of breast cancer and reduction of mortality have been targeted.

Genetic screening in HBOC patients for *BRCA1* and *BRCA2* has been applied for two decades (8). With the increase of the knowledge on cancer genetics and the development in next-generation technologies and multigene panels, recommendation on genetic testing of at-risk patients has been expanded (9, 10). Germline genetic testing

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is recommended for all of the newly diagnosed breast cancer patients because a possible pathogenic mutation is crucial to guide the patient's follow-up period as well as to direct medical and/or surgical treatment options (11). The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommends managing genetic testing for HBOC patients according to individual criteria and evidence (9). Patients with multiple criteria are more likely to have a genetic origin. The highest rate of pathogenic mutations is identified in patients with premenopausal breast cancer and in the patients who have a history of multiple affected individuals in the family (12, 13). Additionally, germline genetic testing also provides early diagnosis and prevention of the disease by determining pre-symptomatic mutation carriers.

In the light of the criteria specified in the international guidelines, it is important for each population to create a genetic testing algorithm according to its own characteristics. Since the Turkish Genome Project is not fully completed, the clinical association of the variations of unknown clinical significance detected in genetic screening is contradictory. Increase in the studies of genetic testing of HBOC patients may provide a better understanding for founder mutations, and expand the mutational spectrum in non-BRCA HBOC genes in the Turkish population.

MATERIALS and METHODS

Patients and samples

Totally, 230 subjects were performed at Numune Hospital and Ankara City Hospital, Medical Genetics Clinic, at 2019. Written informed consent was obtained from all patients before testing for the use of their DNA samples for research purposes. All the patients previously BRCA genes tested for three such groups: women with a personal history of bilateral breast cancer, women with a personal history of breast cancer and a first-degree or second-degree relative with ovarian cancer, and women with a personal history of ovarian carcinoma according to the National Comprehensive Cancer Network (NCCN) guidelines. The patients whose first analyze reported as negative in both next-generation sequencing and Multiplex Ligation-dependent Probe Amplification (MRC-Holland® SALSA® MLPA® probemix P060) tests, accepted for the second test.

Genetic testing

Blood samples were collected into EDTA tubes. DNA of patients extracted by QIAsymphony® automated DNA isolation system (Qiagen Inc. Mississauga, ON, Canada). Hereditary breast cancer (without *BRCA1/BRCA2*) panel (Table 1) was designed with QIAseq (Qiagen, Hilden, Germany) solution and used for sequencing. The sequencing process was performed on the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The data analyses were performed on QIAGEN Clinical Insight (QCI) Analyze software (QIAGEN, Hilden, Germany).

Variant classification

During the variant filtering process, we considered only nonsense and missense variants, indels, and variants at canonical splice sites, whereas variants with minor allele frequency greater than 0.01 in different public and local resources [Exome Sequencing Project (ESP, http://evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) data, 1000 Genomes Project (http://www.1000genomes.org)] were excluded. After the initial filtering process, we followed the guidelines for the interpretation of sequence variants from the joint consensus recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (14). Variants evaluated as pathogenic or probably pathogenic according to the ACMG and AMP criteria were included. The status of the variants checked at The Human Gene Mutation Database (HGMD). Possible pathogenic variants are considered the probable cause of the disease or the effect on the protein function is predicted to be likely deleterious (>90% probability of causing the disease). Variants of uncertain significance (VUS) are genetic variants with unknown or questionable impact on the condition.

RESULTS

In this study, mutations in *BRCA1* and *BRCA2* genes were investigated in 230 patients who applied to our outpatient clinic for breast cancer. Pathogenic / likely pathogenic variants were detected in 25 (10.8%) of these patients, while VUS was detected in 19 (8.2%) of them.

The genes in Table 1 were studied with the new generation sequencing method in 38 of the patients without pathogenic or VUS variants (Figure 1). Pathogenic / likely pathogenic variants were detected in 3 (7.8%) of 38 patients, while VUS was detected in 10 (26%) patients. Of these 22 genes, c.312C>A (p.Tyr90Ter) in MUTY, c.1225C>T (p.Arg409Trp) in *STK11*, c.1690C>T (p.Gln564Ter) variant in *BARD1* interpreted as pathogenic. These changes were also included in the HGMD as 'Disease causing mutation (DM)'. In addition, VUS was detected in 7 of 22 genes (ATM, *BARD1*, BRIP1, CDH1, CHEK2, MRE11A, *MUTYH*, *STK11*). Among these VUS's, c.3149C>A (p.Thr1050Asn) variant in BRIP1 gene with CM179136 (DM?) In HGMD; c.538C>T (p.Arg180Cys) variant in CHEK2 gene with CM030417 (DM) in HGMD; c.470T>C (p.Ile157Thr) variant in CHEK2 gene CM993368 (DFP) in HGMD; c.1496A>G (p.Glu499Gly) variant in MRE11 gene CM160123 (DM?) in HGMD; c.796G> T (p.Val266Phe) variant in the CDH1 gene CM1817928 (DM?) in HGMD (Table 2).

Table 1. Gene content of hereditary breast cancer panel

ATM, BARD1, BRIP1, CDH1, CHEK2, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PIK3CA, PMS2, PMS1, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2

Table 2. Described variants from the study group

Gene	Transcript ID	cDNA change	Protein Change	dbSNP	HGMD	Variant type	Zygosity
MUTYH	NM_001128425.1	c.312C>A	p.Tyr90Ter	rs121908380	CM022646 (DM)	Pathogenic	Het
STK11	NM_000455.5	c.1225C>T	p.Arg409Trp	rs368466538	CM1516525 (DM?)	Likely pathogenic	Het
BARD1	NM_000465.4	c.1690C>T	p.Gln564Ter	rs587780021	CM117928 (DM)	Pathogenic	Het
BARD1	NM_000465.4	c.899C>T	p.Pro300Leu	rs961232989		VUS	Het
BRIP1	NM_032043.2	c.3149C>A	p.Thr1050Asn	rs373040333	CM179136 (DM?)	VUS	Het
BRIP1	NM_032043.2	c.1255C>G	p.Arg419Gly	rs150624408		VUS	Het
BRIP1	NM_032043.2	c.56A>G	p.Tyr19Cys	rs876660880		VUS	Het
MUTYH	NM_001128425.1	c.1609A>G	p.Ile537Val	rs757615745		VUS	Het
ATM	NM_000051.3	c.6742A>G	p.Lys2248Glu	rs1555119232		VUS	Het
CHEK2	NM_007194.4	c.538C>T	p.Arg180Cys	rs77130927	CM030417 (DM)	VUS	Het
CHEK2	NM_007194.4	c.470T>C	p.Ile157Thr	rs17879961	CM993368 (DFP)	VUS	Het
MRE11	NM_005591.3	c.1496A>G	p.Glu499Gly	rs774145193	CM160123 (DM?)	VUS	Het
CDH1	NM_004360.5	c.796G>T	p.Val266Phe	rs1555515463	CM1817928 (DM?)	VUS	Het

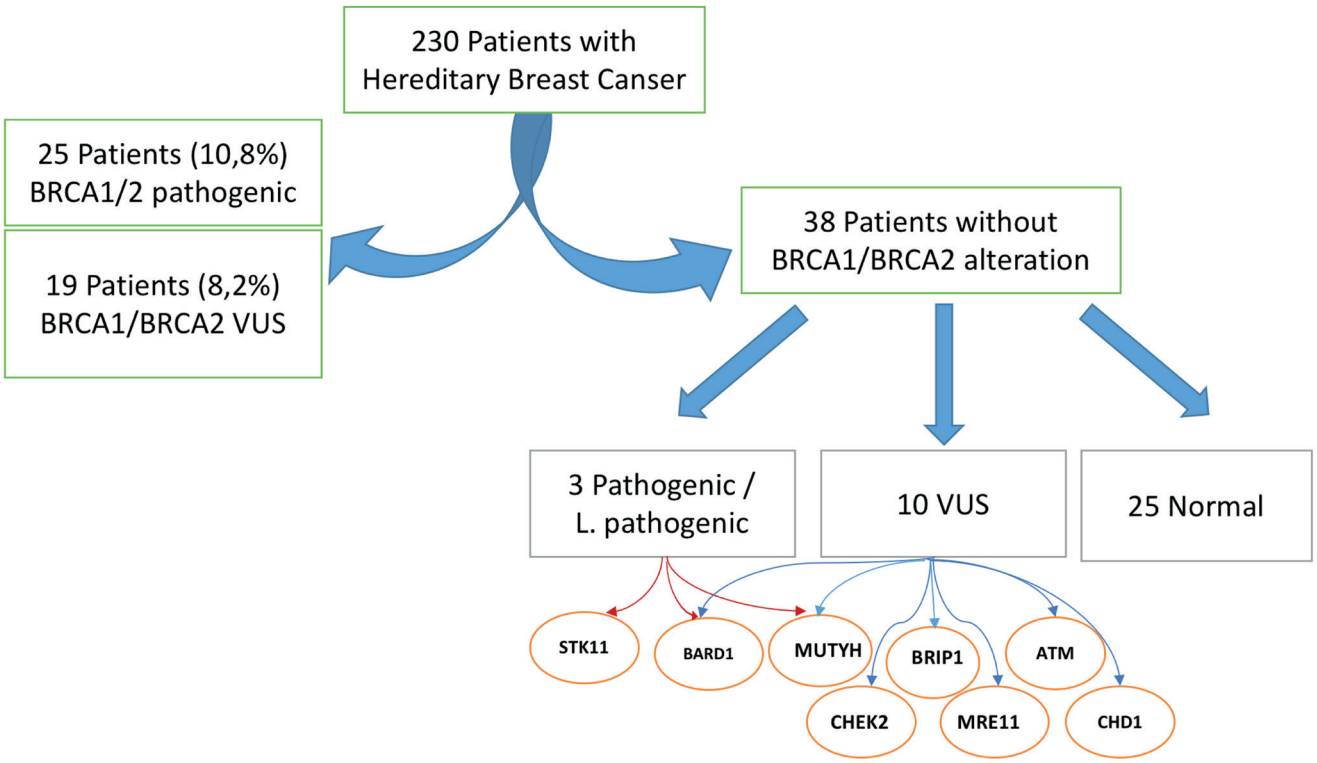

Figure 1. Study design and summary of the approach

DISCUSSION

Breast cancer is a heterogeneous disease defined by several molecular subtypes. Hereditary breast and ovarian cancer syndrome (HBOC) is a hereditary cancer predisposition syndrome, characterized by increased incidence of breast, ovarian cancer and the other solid tissue tumors in the family. Approximately 5%–10% of breast cancers are due to genetic predisposition caused by germline mutations; the most commonly tested genes are *BRCA1* and *BRCA2* mutations (15). Germline mutations in the *BRCA1* and *BRCA2* genes result in hereditary breast and ovarian cancer syndrome. Both BRCA genes are tumor suppressor genes that encode proteins that function in the DNA repairing process. In addition to the widely studied *BRCA1* and *BRCA2*, genetic testing for mutations in other familial breast cancer-associated genes, for instance PTEN, TP53, ATM, CHEK2 and PALB2 using multiple-gene sequencing panels had shown its important clinical values with the advances in next-generation sequencing technology (16).

In this study, we evaluated the patients who applied to the medical genetic clinic retrospectively. 230 patients applied for breast cancer. We accepted patients recommended for study according to The NCCN Clinical Practice Guidelines. Firstly, the *BRCA1* and *BRCA2* genes were sequenced to 230 patients with new generation sequencing and deletion and duplication research was performed in these genes. Pathogenic / likely pathogenic changes were detected in 25 (10.8%) of these patients, while VUS was detected in 19 (8.2%) of them (Figure 1). It is known that frequencies of deleterious variations of *BRCA1* / 2 vary between populations. The prevalence of *BRCA1*/2 in Japan is reported to be 2.6%, while in the US it is as high as 11.1% (4, 17). The prevalence of BRCA mutations and clinical characteristics associated with these mutations in Turkish population has not been well studied. In the recent study conducted in Turkey showed that 9.4% of the pathogenic variants (18). Deletions and duplications of *BRCA1* / 2 are also important and mutations at different rates between 1-3% have been reported. It is emphasized that it is important to investigate copy number variations in the diagnosis of HBOC (19). In our patient group, the rate of deleterious variant detection was similar to the literature.

In 38 of the patients without any changes, sequencing of other genes with HBOC was performed with the new generation sequencing method. Pathogenic / probably pathogenic mutations were detected in 3 of HBOC related genes (*MUTYH*, *STK11*, *BARD1*). *MUTYH* has been associated with one of the DNA mismatch repair system genes and gastrointestinal system malignancies; however the gene has also been shown in breast cancer somatic and germline mutations in recent years (20). *STK11* is associated with Peutz-Jeghers syndrome; its relationship with breast cancer has also been reported. The c.1225C>T (p.Arg409Trp) in *STK11* mutation detected in a patient has been previously described in the study and reported to possible cause breast cancer (21). *BRCA1*-associated RING domain-1 (*BARD1*) predispose to hereditary breast and/or ovarian cancer (22). The c.1690C> T (p.Gln564Ter) variant in the *BARD1* gene early terminates the protein and the relationship between breast cancer has been reported in the literature (16). In addition, the c.899C>T (p.Pro300Leu) variant in the *BARD1* gene was detected in another patient at our cohort. This variant with a genomAD frequency of less than 0.001 was estimated to be VUS since it was not previously associated with the disease. However, in following studies, the structure of the gene may be associated with the disease as it is more understood. The difference between the variant that is reported as pathogenic and the variant that is reported as VUS in *BARD1* is that seriously changes the *BARD1* protein structure.

Different ratios have been found in studies in which HBOC-related genes other than *BRCA1* and *BRCA2* genes have been investigated. The fact that the studied genes are more comprehensive and they have different rates in different populations explain the change in rates. Crawford et al. reported different rates between 5-18% however, Tsaousis et al. reported 22% mutations in the panel containing 36 genes (23, 24). Couch reported 10.2% in a panel containing 21 genes in the study in a large number of patients (15). In a study from Turkey pathogenic variant was 8.5%, respectively (25). In our study with fewer cases, pathogenic / likely pathogenic variants were detected in 3 (7.8%) of 38 patients, while VUS was detected in 10 (26.3%) patients (Table 2). We detected a high rate of VUS, similar to Tsaousis's research. Since the function of the genes in the panels where many genes are studied and their contribution to the etiology of breast cancer is not yet fully elucidated, the rate of VUS is high. It will contribute to the evaluation of patients with the increase of such studies.

CONCLUSION

In order to implement clinical genetic strategies adapted to each population's needs and intrinsic genetic characteristic, this study aims to present the current status of knowledge about the spectrum of HBOC related pathogenic variants in Turkish population.

Our data provide insight into the genetics of HBOC syndrome in Turkey. The screening of HBOC related genes in large cohort of patients will help to know about the frequency, the spectrum, the contribution and the prevalence of the gene mutations. These studies will help to improve the clinical management and better risk assessment of hereditary breast and ovarian cancer.

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