

High-Resolution Melting Analysis for Screening of Turkish Germline Mutations in BRCA1 and BRCA2

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ABSTRACT

The germline mutations of breast cancer susceptibility genes 1 (BRCA1) and 2 (BRCA2) are the two most frequently mutated genes in inherited breast and ovarian cancer. Among the most known mutations in these tumor suppressor genes are 5382insC and 185delAG in BRCA1 and 6174delT in BRCA2. The aim of the current study was to investigate the frequency of these BRCA1 and BRCA2 mutations in Western Turkish population. Twenty-five women with a history of self breast cancer and family history, 25 women with a familial history of breast cancer in their first degree-relatives and five healthy women formed the studied groups. DNA from peripheral blood was extracted and analyzed by high-resolution melting (HRM) analysis. None of the 50 patients and five healthy individuals was found to carry 185delAG mutation in BRCA1 and the 6174delT mutation in BRCA2. But, we found the 5382insC mutation in exon 20 of BRCA1 in five patients, having a strong family history. Four of these five patients were from the same family. Our preliminary results indicate that penetrance of 5382insC mutation in BRCA1 mutations is dominant in Turkish population; however, it seems there might be some other genes that contribute more significantly to familial breast carcinoma in Turkish population in BRCA.

Keywords: BRCA1, BRCA2, Mass screening, Turkish population

ÖZET

Türk Hasta Popülasyonunda BRCA1 ve BRCA2 Germline Mutasyonlarının High Resolution Melting Analizi ile Taranması

Kalıtıl meme ve over kanseri gelişiminde germline mutasyonu en sık rastlanan genler breast cancer susceptibility genes 1 (BRCA1) ve 2 (BRCA2)'dir. Bu tümör baskılayıcı genlerde en sık rastlanan mutasyonlar ise BRCA1 için 5382insC ve 185delAG iken BRCA 2'de 6174delT'dir. Çalışmanın amacı Türkiye'nin batısındaki popülasyonda bu mutasyonların sıklığını kuvvetli aile öyküsü olanlarda saptamaktır. Çalışmaya kendisinde ve birinci derece yakınlarında meme ya da over kanseri öyküsü olan 25 kadın ile kendisinde kanser öyküsü olmadığı halde birinci derece yakınlarında meme ya da over kanseri öyküsü olan 25 kadın dahil edildi. Beş adet sağlıklı kontrol de çalışmaya alındı. Periferik kan örneklerinden izole edilen DNA'lar high-resolution melting (HRM) analizi ile çalışıldı. BRCA1'deki 185delAG ve BRCA2'deki 6174delT mutasyonu ne çalışma ne de kontrol grubunda saptanmadı. Ancak, BRCA1'deki 5382insC mutasyonu dördü aynı aileden olan toplam beş kişide saptandı. Çalışmamızın öncü sonuçları 5382insC mutasyonunun Türk hasta popülasyonunu için daha dominant bir penetrans gösterdiğini saptamakla birlikte, BRCA geninde Türklere özgü yeni dominant mutasyonlarının da belirlenmesi gerekliliğini ortaya koymuştur.

Anahtar Kelimeler: BRCA1, BRCA2, Tarama

INTRODUCTION

Inherited gene mutations are responsible for 5–10% of familial breast cancers and 7-10% of familial ovarian cancers.¹ Breast cancer susceptibility genes 1 (BRCA1) and 2 (BRCA2) are the two most frequently mutated genes in inherited breast and ovarian cancer. Carriers of BRCA1 or BRCA2 mutations have an increased lifetime risk of between 60% and 85% for developing breast cancer, and a lifetime risk of between 26% and 54% for developing ovarian cancer for BRCA1, and between 10% and 23% for BRCA2.²⁻⁶

The number of germline mutations identified within BRCA1 and BRCA2 genes varies widely among populations in the world.⁷ Dominant mutations in these genes are the 5382insC and the 185delAG in BRCA1 and the 6174delT in BRCA2 whereas the 5382insC in exon 20 of BRCA1 is one of the most common mutations in Central and Eastern Europeans (Russian and Turkish populations).⁸⁻¹² This exon also contains the 5331G>A BRCA1 mutation recently shown to be a founder mutation in Greek Europeans.¹³ The 185delAG was also observed in non-Ashkenazi Jews,¹⁴⁻¹⁶ and in non-Jewish individuals from several ethnic backgrounds.^{17,18}

Numerous methods may have been used screening the BRCA1 and BRCA2 genes; foremost direct sequencing, then denaturing gradient gel electrophoresis (DGGE), protein truncation test (PTT), denaturing high performance liquid chromatography (DHPLC) and single-strand conformation polymorphism (SSCP).^{19,20} High-resolution melting (HRM) is a new methodology for mutation scanning in which the mutation scanning is carried out

in the same tube or well in which the sequence is amplified.²¹ The PCR reactions and HRM can all be performed in a single run of less than 2 hours resulting in extremely rapid screening. In the present study, BRCA1 and BRCA2 mutations in 50 breast and/or ovarian cancer patients (from Western Turkey), having a positive family history of breast and/or ovarian cancer were analyzed by HRM analysis to elucidate the genotypical characteristics of Turkish breast cancer patients.

PATIENTS AND METHODS

Patients

Fifty women with family history of breast and/or ovarian cancer in their first degree-relatives were included in this study. Twenty-five of them had also self history of breast cancer. Five healthy women without any self or family history of breast/ ovarian cancer were taken as control group (n= 55). All patients enrolled in the study had admitted during 2008-2009 to Tulay Aktas Oncology Hospital, Medical School of Ege University, Izmir. Ege University is a referral hospital that admits patients from the western provinces of Turkey. All participating women were informed that their DNA samples would be analyzed for genotyping and the informed consents for the use of their DNA were signed. The protocol was approved by the Ethical Committee of the Ege University of Medical Sciences.

PCR and HRM Analysis Conditions

Blood samples from patients were collected and DNA extraction was carried out in MagNA Pure

Table 1. List of primers used to amplify BRCA1 exon 2, BRCA1 exon 20 and part of BRCA2 exon11.

Gene		Sequence
BRCA1 exon 2	Forward primer	5'-AAAAGATATAGATGTATGTTTTGCTAATGTGT-3'
	Reverse primer	5'-TCCCAAATTAATACACTCTTGTGCTGA-3'
BRCA1 exon 20	Forward primer	5'-GAGTGGTGGGGTGAGATTTTTGTC-3'
	Reverse primer	5'-CCTGATGGGTTGTGTTTGGTTTCT-3'
BRCA2 exon 11	Forward primer	5'-CGAAAATTATGGCAGGTTGTTACG-3'
	Reverse primer	5'-GCTTCCACTTGCTGTACTAAATCCA-3'

Table 2. Frequency of BRCA1 and BRCA2 mutations.

Phenotype Patients	Number of patients	BRCA1 mutations	BRCA2 mutations	Ratio (%)
Breast cancer cases with a history of breast cancer in their first degree relatives	25	1	0	1/25 (0.04)
Breast cancer cases with a history of both breast and ovarian cancer	2	1	0	1/2 (0.5)
Breast cancer cases with ovarian cancer history in their first degree relatives	3	0	0	0/3 (0)
Individuals with no sign of breast cancer with breast/ ovarian cancer history in their first degree relatives	25	3	0	3/25 (0.12)
Total	50	5	0	5/50 (0.1)
Early-onset cases				
20-35 years	5	2	0	2/5 (0.4)
36-60 years	20	3	0	3/20 (0.15)
Total	25	5	0	5/25 (0.2)

Compact Nucleic acid Isolation Instrument by using MagNA Pure LC DNA Isolation Kit I (Roche Applied Science, Germany), according to the manufacturer's instructions. DNA quality was measured with Nanodrop ND1000 Spectrophotometer (Nanodrop Technologies, USA). Primers used to amplify BRCA1 exon 2, BRCA1 exon 20 and part of BRCA2 exon11 were synthesized by TibMolBiol (Berlin, Germany) (Table 1).

The PCR and HRM were performed in LightCycler® 480 instrument (Roche Diagnostics, Penzberg, Germany) in a reaction mix containing 25 ng of genomic DNA, either 400 nM (exon 2 and exon 11) or 200 nM (exon 20) of each primer and 3 mM MgCl₂ in the LightCycler® 480 HRM Master containing ResoLight dye (Roche Diagnostics) with PCR grade water adjusted to a total volume of 20 µl. The reaction condition included an activation step at 95°C for 10 minutes followed by 55 cycles of 95°C for 10 seconds, a touch down of 65°C to 55°C for 10 seconds (1°C/cycle) and 72°C for 30 seconds. Before the high-resolution melting step the products were heated to 95°C for 1 minute. The HRM was carried out over the range from 72°C to 95°C rising at 1°C per second with 30 acquisitions per degree. All reactions were performed in duplicate.²²

HRM Analysis

Upon completion of the run (approximately 2 hours), HRM curve analysis was performed using the LightCycler® 480 Software version 1.3. supplied with the LightCycler® 480 instrument. The melting curves were normalized, to allow samples to be directly compared, temperature was shifted. Difference plots were generated by selecting a negative control, converting the melting profile to a horizontal line and normalizing the melting profiles of the other samples against this sample. Significant differences in fluorescence from the horizontal baseline were indicative of mutations. Differences were judged as significant if the replicates fell outside the range of variation seen in the wild type samples.²²

RESULTS

The mean age of fifty patients is 43.9 ± 7.3 years, while mean age of five unrelated healthy women is 41 ± 4.7 years. We investigated the presence of BRCA1 and BRCA2 mutations in 50 patients with a personal and/or family history of breast and/or ovarian cancers, and five healthy patients from western Turkey by HRM analysis. Twenty-five of the 50 patients in this group had breast cancer with

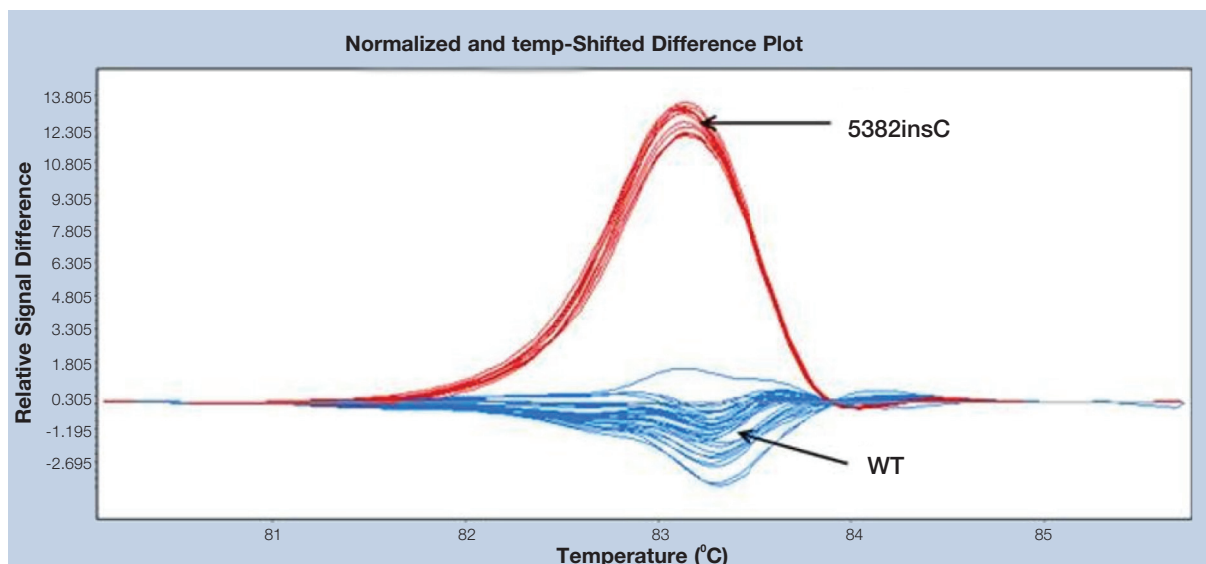


Figure 1. Difference plot showing the mutation relative to the wild type controls. In difference plots, the melting profile of a wild type control is chosen as a horizontal base line and the relative differences in the melting of all the other samples are plotted relative to this baseline. The figure shows difference plots for the exon containing one of the most common middle-east mutations (5382insC). Each trace represents the amplicon from a different individual's DNA sample. All mutations were clearly distinct from the wild type controls.

a history of breast cancer in their first degree relatives. Two of the twenty-five patients had history of ovarian cancer in addition to breast cancer history. Three of these twenty-five patients had ovarian cancer history in their first degree relatives. Twenty-five of 50 patients had no sign of breast cancer; however, had a breast cancer history in their family whereas five had an additional history of ovarian cancer in the family. The total DNA from five unrelated healthy controls was also tested to confirm the absence of the identified mutations in the normal population. Positive controls of known BRCA mutant genotypes were also used in all PCR reactions.

None of the 50 patients and five healthy individuals was found to carry 185delAG mutation in BRCA1 and the 6174delT mutation in BRCA2. We only found the 5382insC mutation in exon 20 of BRCA1 in five patients, having a strong family history. Four of these patients were from the same family and one out of this family had both breast and ovarian cancer history. Details of family history and cancer onset ages are summarized in Table 2. We found mutations in five of 50 patients, thus bringing the total contribution of BRCA1 to 10% in our study

population. The remaining patient out of five with positive mutation had history of ovarian cancer and family history of breast cancer.

DISCUSSION

In the present study BRCA1 and BRCA2 mutations were investigated in Turkish breast and ovarian cancer patients using HRM analysis. Fifty cases with a family history of breast and/or ovarian cancers, three BRCA1 and one BRCA2 mutations have been searched. The 5382insC and the 185delAG mutations in BRCA1 and the 6174delT in BRCA2 occur in the general Ashkenazi Jewish population with a carrier frequency of 1.09% for the 185delAG mutation, 0.13% for the 5382insC mutation, and 1.52% for the 6174delT mutation.²³

There are several studies reporting these founder mutations in other populations.²⁴⁻²⁶ Of these, 5382insC mutation in BRCA1 was previously shown in Turkish populations with a breast cancer family history. In consistent with the previous knowledge, 5382insC mutation in BRCA1 were also detected in our study group, indicating the fact that this mutation has a dominant penetrance for in-

herited breast and ovarian cancer cases in Turkey (Figure 1). It is also noteworthy to mention that two out of five mutational cases had very early (<30 years) onset of breast cancer.

Combined BRCA1 and BRCA2 germline mutations are responsible for important proportion of all hereditary breast cancer cases (2% to 5% of all incident breast cancer cases).²⁷⁻²⁹ Furthermore, mutations in both genes contribute to early onset breast cancer: 6% to 16% of all breast cancer cases diagnosed before the age of 36 years are predicted to carry a BRCA1 mutation and a somewhat lower or similar percentage a BRCA2.³⁰ In our study, both of the 6174delT and the 185delAG mutations were not detected either in 50 patients or in five healthy individuals. Based on this fact, in Turkish population with a family history of breast/ ovarian cancer, these mutations in BRCA1 /2 cannot be accepted as good indicators to search for germline mutations. So, it seems logical that Turkish high risk individuals should first be screened for the 5382InsC BRCA1 mutation before full analysis of both genes is carried out.

In conclusion, it seems that penetrance or prevalence of BRCA1 mutations-specifically 5382insC mutation- is about 10% in Turkish population, which is parallel with the literature. But, based on the results of this preliminary research, it seems there might be some other genes that contribute more significantly to familial breast/ovarian carcinoma in Turkish population in BRCA.

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