Identification of a BRCA2 mutation in a Turkish family with early-onset breast cancer

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CASE REPORT

INTRODUCTION

Breast cancer is the most common and deadly cancer among women. The vast majority of breast cancers are sporadic, arising from somatic mutations, whereas 10% of all breast cancer cases are hereditary, clustering in families and having an early onset. Deleterious germline mutations in BRCA1 and BRCA2 genes are the most important risk factors for hereditary breast and ovarian cancer, which is inherited in an autosomal dominant manner. Individuals with these germline mutations have a lifetime risk of developing breast cancer of 50-80%.

Key Clinical Message

We used a multi-gene panel testing to identify the germline variants in a mother-daughter pair with early-onset breast cancer, and detected one pathogenic protein-truncating variant in BRCA2. Our results highlight the importance of genetic testing in identifying the pathogenic mutation running in cancer families.

Keywords

ATM, BRCA1/2, early-onset breast cancer, genetics, multi-gene panel testing, oncology
up to 25% of all familial breast cancers. Additional cancer predisposition genes (e.g., ATM, PALB2, TP53, BARD1, CHEK2) are also implied in hereditary breast cancer.

Most breast cancer predisposition genes have functions in maintaining genome integrity and cell cycle control. ATM, a serine/threonine protein kinase, functions as a transducer of DNA damage signals, and activates downstream proteins, including BRCA1 and BRCA2, by phosphorylation. BRCA1 is an E3 ubiquitin protein ligase and a transcriptional activator. It plays a central role in coordinating cellular pathways in response to DNA damage. Most notably, BRCA1 stimulates DNA repair mechanisms, and arrests cell cycle progression to ensure that DNA is repaired before division. BRCA2 is an ssDNA binding protein, and has a vital role in DNA damage response by regulating homologous recombination.

Genetic tests are recommended for individuals suspected to have germline variants. The results of these tests might be important for personalizing the management of the disease and take preventative measures for the subjects and their families. With multi-gene panel testing, germline variants in the exons of many cancer predisposing genes can be screened in a run in a cost-effective manner. In this case report, we studied a Turkish mother-daughter pair with early-onset breast cancers using a multi-gene panel and identified two variants of uncertain significance (VUS) in ATM, BRCA1 and a pathogenic variant in BRCA2.

2 | MATERIALS AND METHODS

The index patient (the daughter) was recruited in the Surgical Oncology Department of Umreniy Teaching and Research Hospital (UEAH), Istanbul in 2015. Blood samples from the index and her mother, who was also found to have had early-onset breast cancer, were collected and subsequent genetic tests were performed in the joint Genomic Laboratory (GLAB) of UEAH and Istanbul Technical University. Genomic DNAs were isolated using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific) from blood samples of the index patient and her mother. DNA libraries were prepared using TruSight Cancer Kit (Illumina), and sequenced in MiSeq sequencer using 2 × 150-bp paired-end reads. Sequence assembling and variant calling were done using Sophia DDM software (Sophia Genetics). SIFT, PolyPhen-2, MutationTaster, Provean, and Mutation Assessor tools were also used to predict the pathogenicity of missense variants. The family history of cancer was interrogated by medical geneticists at UEAH (Figure 1). The study was carried out with the given consent of the patients.

| Tumor type                                      | Histological grade                      | Nuclear grade            | Tumor localization | ER/PR/cERB2 |
|------------------------------------------------|-----------------------------------------|--------------------------|--------------------|-------------|
| Right breast                                    | Invasive breast carcinoma with extensive in situ components | 3+2+1:6 II/III (Bloom & Richardson modified) | II/III (Black modified) | Lower outer quadrant | −/−/+     |
| Left breast                                     | Invasive breast carcinoma with micropapillary differentiation | 2+2+1:5 II/III (Bloom & Richardson modified) | II/III (Black modified) | 6 o’clock position | +/+/+     |

**FIGURE 1** Pedigree of the index patient with early-onset breast cancer

**TABLE 1** Pathological information of index
and the approval of the ethical committee of UEAH (No: 49/24.03.2016).

3 | RESULTS

The mother-daughter pair studied in this case report developed breast cancers at young age, suggesting an underlying germline mutation. The daughter was diagnosed with bilateral triple negative invasive ductal carcinoma at the age of 33 in 2015, and had bilateral modified radical mastectomy. Cancer has metastasized to the brain; and the patient is currently receiving chemotherapy. The mother was diagnosed with breast cancer at the age of 40 in 2006, had unilateral mastectomy, and is currently alive. Both patients were referred for genetic testing. Pathological information of the index is described in Table 1.

Three germline variants, common to both patients, were detected using a multi-gene panel. The variants in ATM (NM_000051.3c.8965C>G;p.Gln2989Glu) and BRCA1 (NM_007294.3c.3424G>C;p.Ala1142Pro) cause nonsynonymous amino acid changes. The clinical significance of these variants is not known. Further analysis is needed to assess their pathogenicity. On the other hand, the variant in BRCA2 (NM_000059.3c.7655_7658delTTAA:p.Ile2552Thrfs) causes a premature stop codon, leading to the truncation of the C-terminal 866 amino acids in BRCA2 protein. Truncating variants in BRCA2 are highly pathogenic for breast cancer. Variants in BRCA1 and BRCA2 genes were also confirmed by Sanger sequencing.

4 | DISCUSSION

The germline variant in ATM (8965C>G) results in the change of glutamine to glutamic acid at position 2989, which is located in the FATC domain of ATM.11 FATC domain is the binding region for Tip60. The interaction between ATM and Tip60, is important for the activation of ATM.12 In silico prediction tools (SIFT, PolyPhen, MutationTaster, Provean, Mutation assessor) showed conflicting interpretation for this variant.

The variant in BRCA1 results in the change of alanine to proline at position 1142, which is not located in any known functional domain. Proline leads to rigid turns in protein secondary structures, hence missense variants including proline might affect protein folding and structure. However, in silico prediction tools showed conflicting interpretation for this variant.

TTAA deletion in exon 16 of BRCA2 (NM_000059.3c.7655_7658delTTAA:p.Ile2552Thrfs) causes a frameshift starting from codon 2552 and leading to a stop codon after adding 95 amino acids. The wild-type BRCA2 protein is 3418 amino acid long. Thus, this variant is predicted to be highly pathogenic. The resulting truncated protein would be devoid of very important functional domains, including the SEM1-binding site, DNA-binding site, nuclear localization signal (NLS), and the CDK phosphorylation site at S3291, which also binds RAD51.2,13 SEM1 stabilizes BRCA2 by preventing its degradation. In vitro studies showed that loss of SEM1 binding to BRCA2, or depletion of either protein, led to hypersensitivity to DNA damage.13 DNA-binding site is responsible for binding of BRCA2 to single-stranded DNA and acting as a junction between single-strand and double-strand DNA to manage Rad51-mediated homolog recombination.14 Deletion of NLS site causes aberrant localization of BRCA2, preventing its function in maintaining the integrity of DNA and leading to carcinogenesis.15 RAD51 directly binds ssDNA and recruitment is provided by BRCA2. RAD51 binding to BRCA2 at the C-terminus is dependent on the phosphorylation of serine at 3291 by CDK.2

Premature termination codons (PTCs) introduced by BRCA2 mutations also causes degradation of the BRCA2 mRNA by nonsense-mediated mRNA decay (NMD), a protective mechanism that prevents the expression of truncated proteins. PTC-containing BRCA2 transcripts are significantly less prevalent than their counterparts. Therefore, NMD mechanism recognizes PTCs in BRCA2 transcripts and leads to their degradation.16 Reduced BRCA2 levels are associated with cancer, as loss of either BRCA allele is frequently observed in breast cancer tumors of BRCA1 and BRCA2 mutation carriers.2

BRCA2 variant 7655_7658delTTAA was previously reported in the ClinVar database in Polish, Chinese and New

| In situ components | Lenf | T | N | M | V | R | L |
|--------------------|------|---|---|---|---|---|---|
| 90% high grade w/ & w/o necrosis | 35/48 carcinoma metastasis, 13/48 reactive hyperplasia | Pt1a | N3a | X | 0 | 0 | 1 |
| 75% high grade w/ & w/o necrosis | 1/10 carcinoma metastasis, 9/10 reactive hyperplasia | Pt1b | N1a | X | 0 | 0 | 1 |
Zealander breast cancer patients.\textsuperscript{17,18} Now, we report that this pathogenic variant is also found in a Turkish breast cancer family. Deleterious BRCA2 variants also predispose to ovarian cancer, and might occur in families with Fanconi anemia. However, we did not find any member of the family with these diseases. Furthermore, no other breast cancer case was found in the maternal side of the family, suggesting that the pathogenic BRCA2 variant might be a \textit{de novo} germline mutation in the mother of the index. However, we could not receive the consent of the maternal aunts of the index to test this.

Apart from the pathogenic BRCA2 variant, both the index and her mother carry the same two VUS in \textit{ATM} and \textit{BRCA1}. Hence, we cannot infer the clinical significance of these VUS on the basis of the limited data we have. Strikingly, the consanguineous family presented here has an aggregation of lung cancers (Figure 1). Two paternal uncles of the index had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Despite that the uncles had lung cancer; and the paternal cousin and the maternal aunt carry the same two VUS in \textit{ATM} variant in the mother of the index. Apart from the pathogenic BRCA2 variant, both the index and her mother carry the same two VUS in \textit{ATM} and \textit{BRCA1}. Hence, we cannot infer the clinical significance of these VUS on the basis of the limited data we have. Strikingly, the consanguineous family presented here has an aggregation of lung cancers (Figure 1). Two paternal uncles of the index had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Despite that the uncles had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Strikingly, the consanguineous family presented here has an aggregation of lung cancers (Figure 1). Two paternal uncles of the index had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Despite that the uncles had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Moreover, the index had breast cancer, and her mother carry the same two VUS in ATM and BRCA1. Hence, we cannot infer the clinical significance of these VUS on the basis of the limited data we have. Strikingly, the consanguineous family presented here has an aggregation of lung cancers (Figure 1). Two paternal uncles of the index had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Despite that the uncles had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer. Strikingly, the consanguineous family presented here has an aggregation of lung cancers (Figure 1). Two paternal uncles of the index had lung cancer; and the paternal cousin and the maternal uncle had early-onset lung cancer.

In conclusion, in this case report we provide another evidence for the pathogenicity of truncating germline BRCA2 variants in breast cancer.

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**CONFLICT OF INTEREST**

None declared.

**AUTHOR CONTRIBUTION**

EC: and IMA: wrote the manuscript. KET: collected the data for both mother and daughter. GAS: did the experiments. FE: performed the surgery of daughter. GDD: and LD: edited the manuscript.

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