

Double Heterozygosity in the *BRCA1/2* Genes in a Turkish Patient with Bilateral Breast Cancer: A Case Report

Neslihan Duzkale^{1,*} and Nilnur Eyerci²

¹Department of Medical Genetics, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey

²Department of Medical Biology, Faculty of Medicine, Kafkas University, Kars, Turkey

*Corresponding Author: Neslihan Duzkale. Email: neslihanduzkale@gmail.com

Abstract: *BRCA1* and *BRCA2* tumor suppressor genes are responsible for a quarter of hereditary breast cancers. Double heterozygous (DH) pathogenic variant carrier status in these genes is an extremely rare condition, especially in non-Askenazi individuals. We report a woman patient with bilateral breast cancer that carries DH disease-causing variants in *BRCA1/2* genes. The 45-year-old patient who was followed up with the diagnosis of metachronous bilateral breast cancer was diagnosed with cancer at the age of 39 and 43, respectively. *BRCA1/2* genes of the patient were evaluated using Next-Generation Sequencing. In the patient, the c.2800C>T (p.Gln934Ter) pathogenic variant in *BRCA1* and the c.9648+1G>C likely pathogenic variant in *BRCA2* were detected as DH. Segregation analysis in family members revealed that her two healthy siblings available for testing were heterozygous for either *BRCA1* or *BRCA2* variants, but her mother, who had a past diagnosis of ovarian cancer, was heterozygous for both *BRCA1* and *BRCA2* variants. Germline double heterozygosity in inherited cancer is a rare condition, and as far as we know it is reported for the first time from patient population in Turkey. Large-scale patient series are needed to determine the impact of double heterozygosity on diseases course, such as prognosis and treatment responses.

Keywords: *BRCA1*, *BRCA2*; double heterozygosity; hereditary breast and ovarian cancer; bilateral breast cancer

1 Introduction

Only 5–10% of all breast cancers are inherited, and 25% of those are caused by variants in *BRCA1* or *BRCA2* genes. *BRCA1* and *BRCA2* are tumor suppressor genes localized at chromosomal regions 17q21.31 and 13q13.1, respectively, and play an important role in maintaining genomic integrity [1]. These genes are inherited in autosomal dominant manner and they cause *BRCA1*- and *BRCA2*- associated hereditary breast and ovarian cancer syndrome (HBOC). In individuals with HBOC, the risk of developing breast cancer (BC) and ovarian cancer (OC) is significantly increased compared to the general population risk. In individuals with HBOC, there is an increased risk of developing cancer in breast, ovary, prostate, pancreas, skin, and some other organs [2]. According to the results of one meta-analysis, the average cumulative risk of BC in mutation carriers up to the age of 70 has been reported as greater than 50% in *BRCA1* mutation carriers and almost 50% in *BRCA2* mutation carriers. The calculated cumulative risk of OC is 40% for *BRCA1* and 18% for *BRCA2* [3]. In the literature, it has been shown that women who carry *BRCA1/2* mutation have a higher risk of developing bilateral breast cancer (BBC) compared to those who do not [4]. Although approximately 1: 500 individuals in the general population are thought to carry pathogenic variants in *BRCA1* or *BRCA2*, it is extremely rare for the same individual to carry disease-causing variants in both *BRCA1* and *BRCA2* [2]. In this study, we describe a case with metachronous BBC who carries disease-causing variants in both *BRCA1* and *BRCA2* in DH state.



2 Subjects and Methods

2.1 Patient Description

Our patient, who was followed up with a diagnosis of BBC at the age of 45, visited the doctor at the age of 39 due to a palpable mass in her left breast. The pathologic examination has shown that it was negative for estrogen/progesterone receptors and positive HER2 amplification; a diagnosis of invasive ductal carcinoma was made. Clinical staging was determined as T2N0M0. Adjuvant chemotherapy and radiotherapy were administered to the patient after modified radical mastectomy and axillary dissection. Four years after the patient's initial diagnosis of BC, in her right breast, a new mass was detected at routine controls. The pathologic examination of this mass revealed a triple-negative invasive ductal carcinoma, and it was reported as the second primary cancer. TNM staging of this new cancer was also determined as T2N0M0. Modified radical mastectomy and chemotherapy were administered to the patient. The patient was referred to us to determine the genetic etiology predisposing to these cancers.

2.2 Genetic Analysis

Germline variants of the *BRCA1/2* genes were investigated using Next-Generation Sequencing method from DNA obtained from individuals' peripheral blood samples. This study was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) and using the Qiagen Human *BRCA1* and *BRCA2* panel kit (Qiagen, Hilden, Germany). In addition to the exonic regions, intron regions that approximately 20 base pairs on the exon-intron boundary were also evaluated. QIAGEN Clinical Insight (QCI™) Analyze software (Qiagen, Hilden, Germany) was used for bioinformatics analysis of the data. The algorithms suggested in the ACMG guidelines for the classification of sequence variants [5].

3 Results

By genetic analysis, NM_007294.3(*BRCA1*):c.2800C>T (p.Gln934Ter) pathogenic variant and NM_000059.3(*BRCA2*):c.9648+1G>C likely pathogenic variant were detected in the patient. Both variants were not observed in healthy controls in population databases. *BRCA1* c.2800C>T (p.Gln934Ter) variant is predicted to cause truncation or loss of *BRCA1* protein. It has been suggested in the literature that this variant, which has been reported in many individuals affected by breast and/or ovarian cancer, might be a founder mutation in the Japanese population [6–8].

The *BRCA2* c.9648+1G>C variant affects the splice site in intron 26 of the *BRCA2*. This variant is predicted to disrupt the structure of the *BRCA2* protein as a result of its effect on the donor site. Although not reported in people with *BRCA2*-related disease, functional studies have shown that this variant causes loss of function [9].

The family tree of the patient revealed that it was seen that her mother was diagnosed with OC at the age of 55. Her uncle was diagnosed with kidney cancer at the age of 65 and died of his cancer at the age of 73. Her grandfather's sister died at the age of 52 from endometrial carcinoma. There was no cancer in the patient's father's family. Segregation analysis was performed to investigate the presence of these variants in the family.

The *BRCA2* c.9648+1G>C variant was detected in healthy sister and the *BRCA1* c.2800C>T variant was detected in healthy brother. Her mother was DH for both these variants. Segregation analysis could not be performed on the other family members (Fig. 1).

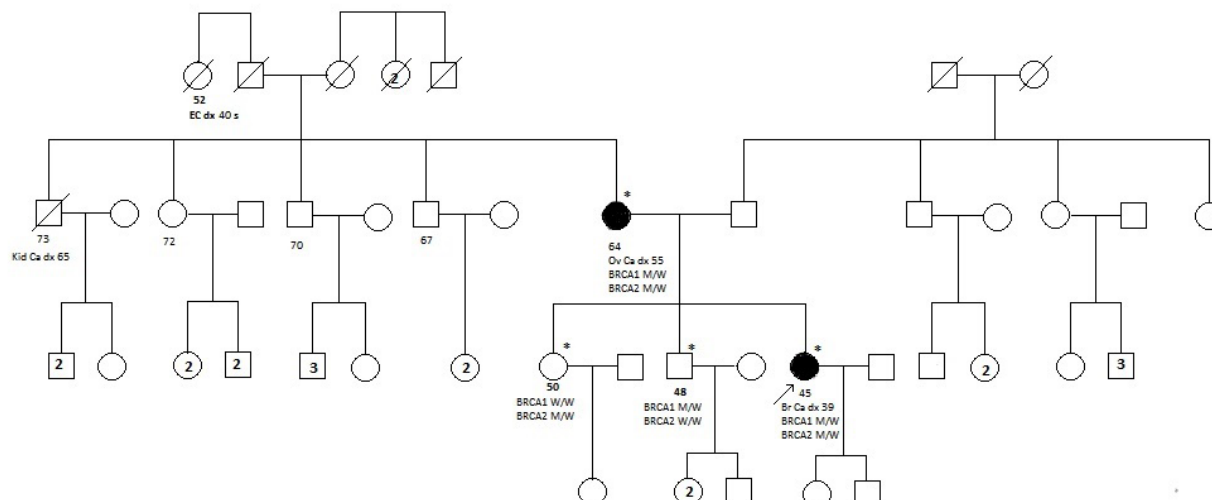


Figure 1: Family tree. The proband is indicated with an arrow. Individuals undergoing genetic analysis are indicated by an asterisk (*). The age, genotype and phenotype are listed below the symbols. Square symbol: Male, Circle symbol: Female, EC: Endometrium Cancer, Ov Ca: Ovarian Cancer, Kid Ca: Kidney Cancer, Br Ca: Breast Cancer, dx: Age at diagnosis, M/W: Heterozygous genotype, W/W: Wild genotype

4 Discussion

There are few publications in the literature investigating individuals carrying the *BRCA1* and *BRCA2* variants as DH. The first of these was the study with Ashkenazi Jewish patients with breast and/or ovarian cancer. Researchers have reported that common mutations in the Ashkenazi-Jewish population were carried as DH in some of the patients [10]. In the Ashkenazi population, the incidence of pathogenic variants in *BRCA1* and *BRCA2* was 2.5%, and 0.3% of women with BC in this population were found to have DH [11,12]. In one study in the literature, the cumulative cancer incidence rate for BC in individuals with DH up to the age of 70 was found to be 80%. In the same study, the incidence of DH was reported as 0.22–0.87% among *BRCA1/2* mutation carriers and 1.8% in the Ashkenazi-Jewish population. No evidence was found in this publication that DH women have an increased risk of multiple primary malignant neoplasias (MPMN) [13]. In another study, the incidence of DH was reported to be 1 in 1800 and 1 in 190,000 in the Ashkenazi and non-Ashkenazi population, respectively [14]. In studies conducted in various populations, it has been determined that the cumulative incidence rate for BC and OC is higher in DH carriers than population-based data [15,16]. One study reported that Caucasian female DH carriers were not younger than patients with a single mutation at the time of diagnosis, and these cases had a more severe cancer phenotype than their single heterozygous relatives [17]. In another study, 4 female patients with DH developed mostly triple-negative BC, and three of them also developed OC. The mean age at diagnosis of BC and OC were 42.7 and 48.6 years, respectively, and patients showed clinically poor prognosis [18]. Although there are no large case series of DH cases in the literature, some publications report that these individuals are more likely to be diagnosed with cancer and are more prone to MPMN [19,20].

Our patient, who has been identified with *BRCA1/2* DH pathogenic variants after the diagnosis of BBC, received the first BC diagnosis at an early age (39 years). The second primary cancer focus was detected in the other breast of the patient 4 years later. There are few studies in the literature regarding the risk of developing BBC in these individuals. Publications have reported that the risk of BBC in *BRCA1* or *BRCA2* mutation carriers is 32–64%. The risk of developing second BC in these carrier women was 13–40% within 10 years after the first BC diagnosis. This increased risk has been reported as 24% in *BRCA1* carriers and 19% in *BRCA2* carriers. The development time of secondary BC has been reported as 5.1 years in *BRCA1* carriers and 5.2 years in *BRCA2* carriers [4,21]. Prolonging the life expectancy of patients due to the advances in diagnosis, screening technology and the increase in the success rates of treatment methods for BC patients may lead to an increase in the incidence of bilateral breast cancer.

Reasons such as BC type in lobular histology, gene mutations, early diagnosis of BC, and a history of exposure to radiation in previous cancer treatment may also lead to increase BBC risk [22,23]. The fact that our patient received radiotherapy for the first BC treatment may have contributed to the etiopathogenesis of the BBC. Also, DH status may play a synergistic role in the carcinogenesis process by causing a decrease or loss of the tumor suppressor function of *BRCA1/2*. This situation can be clarified by investigating the large case series.

Performing *BRCA1/2* genetic analysis of patients with a diagnosis of BC at ≤ 45 years old, as recommended in the NCCN guidelines, will guide the individuals for cancer screening, surveillance, prevention, and treatment programs [24]. In cancer predisposition syndromes, it is important to recommend segregation analysis in families after identifying high-risk patients. Performing cosegregation analysis in relatives of patients with disease-causing variants enables the identification of carrier relatives. In our study, the mother of the proband who was followed up with a diagnosis of OC was found to be a double heterozygous carrier, and the two healthy siblings of the proband were found to be heterozygous for a variant in a single gene. Genetic counseling on HBOC and increased cancer risk were provided to both the patient and her family. Informing HBOC cases about the cancer risk caused by highly penetrant pathogenic variants in *BRCA1/2* genes is important to raise awareness of them and their relatives. In HBOC, large-scale studies including multiple cases who are DH carriers of *BRCA1/2* variants will provide clarification of the genotype-phenotype association in these cases.

Compliance with Ethical Standards: Before the study, written informed consent was obtained from the patient and her relatives who underwent genetic analysis for the publication of this case report and accompanying images. This case study was approved by the independent Ethics Committee of Ankara Dışkapı Yıldırım Beyazıt Training and Research Hospital (Document No. 2020-86/09). The present study involved human participants, and it was conducted considering ethical responsibilities according to the World Medical Association and the Declaration of Helsinki.

Author Contributions: ND: Principal investigator, study conception and design; ND, NE: Sample collection and molecular analysis; ND: Patient management and interpretation of data. All authors were involved in drafting the article and/or revising it critically for important intellectual content, and all authors approved the final version to be published.

A Data Availability Statement: The data are available on special request.

Acknowledgment: The authors thank the patients who agreed to be included in this study.

Funding Statement: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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