Original Article

Investigation of BRCA2 Gene K3326X Variant in Patients with Breast and Ovarian Cancer by Next-Generation Sequencing Technique

Yeni Nesil Dizileme Tekniği ile BRCA2 Geninde K3326X Varyantı Tespit Edilen Meme ve Over Kanseri Hastalarının İncelenmesi

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ABSTRACT

Introduction: The BRCA2 gene is a tumor suppressor gene involved in the repair of double-stranded DNA damage by homologous recombination. Until now, many cancer-related variants of the BRCA2 gene have been reported. There are conflicting publications in studies of the possible effect of the K3326X variant of this gene in cancer. This study investigates the K3326X BRCA2 gene variant and its role in the cancer pathogenesis of Turkish patients diagnosed with breast and ovarian cancer.

Materials and methods: In the study, 1957 patients with cancer diagnosis for BRCA1 and BRCA2 genetic analysis and 432 healthy individuals without a history of cancer were included. The K3326X variant was investigated using the next-generation sequencing method from the genomic DNA sample obtained from the individuals.

Results: K3326X variant was detected in 54 of 1957 (2.75%) cancer patients. For the non-cancerous group, 11 of 432 (2.5%) patients were carrying the K3326X variant. When both groups were compared in terms of K3326X variant carriage, a statistically significant result could not be obtained for the individuals (p=0.934).

Discussion: BRCA2 K3326X variant did not have a significant role in cancer etiopathogenesis. As a result, the variant whose clinical significance is not still been fully understood, was investigated for the first time for Turkish population. Our results suggest that the variant could be a benign variant.

Keywords: BRCA2, Breast cancer, Ovarian cancer, K3326X

ÖZET

Giriş: BRCA2 geni, homolog rekombinasyon ile çift sarmallı DNA hasarının onarımında yer alan bir tümör baskılayıcı gendir. Şimdiye kadar, BRCA2 geninin kanserle ilgili birçok varyantı rapor edilmiştir. Bu genin K3326X varyantının kanserdeki olası etkisine ilişkin çalışmalarda çelişkili yayınlar bulunmaktadır. Bu çalışmada meme ve yumurtalık kanseri tanısı almış Türk hastaların K3326X BRCA2 gen varyantı ve kanser patogenezindeki rolü araştırılmıştır.

Gereç ve yöntemler: Çalışmaya BRCA1 ve BRCA2 genetik analizi için kanser tanısı konan 1957 hasta ve kanser öyküsü olmayan 432 sağlıklı birey dahil edildi. Bireylerden elde edilen genomik DNA örneğinden yeni nesil dizileme yöntemi kullanılarak K3326X varyantı araştırıldı.

Bulgular: 1957 kanser hastasının 54'ünde (%2.75) K3326X varyantı tespit edildi. Kanserli olmayan grup için 432 hastanın 11'i (%2,5) K3326X varyantını taşıyordu. Her iki grup K3326X varyant

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taşıyıcılığı açısından karşılaştırıldığında, bireyler için istatistiksel olarak anlamlı bir sonuç elde edilemedi (p=0,934).
Tartışma: Çalışmamızda BRCA2 K3326X varyantının kanser etyopatogenezine anlamlı etkisi tespit edilememiştir. Sonuç olarak, klinik önemi henüz tam olarak anlaşılamayan bu varyant, ilk kez Türk popülasyonu için araştırılmıştır. Sonuçlarımız, varyantın benign bir varyant olabileceğini düşündürmektedir.

Anahtar kelimeler: BRCA2, Meme kanseri, Over kanseri, K3326X

Introduction

BRCA2 gene is one of the important tumor suppressor genes that play a role in maintaining genomic stability in breast cancer. This gene was determined as Fanconi anemia (FA), complementation group D1 (FANCD1), which helps to repair DNA double-strand breaks by its homologous recombination mechanism. This powerful tumor suppressor gene is responsible for the formation of the BRCA2 protein, which is actively involved with some other proteins in many processes such as cell cycle and DNA replication control, telomere hemostasis, and maintenance of genomic integrity [1]. BRCA2 is one of the genes responsible for hereditary breast and ovarian cancer syndrome (HBOC), and its germline disease-related variants cause susceptibility to certain types of cancer such as; breast, ovarian, and prostate in the individual, and show intermediate penetrance (20–50%) in terms of cancer [2]. Meta-analysis results have shown that in carriers of pathogenic variants of the BRCA2 gene, the mean cumulative risk increase in female sex up to age 70 is 38-84% for breast cancer and 16.5 -27% for ovarian cancer. Male BRCA2 pathogenic variant carriers were estimated to have breast cancer risk by 6.8%, and lifetime risk of prostate cancer is 20% up to the age of 70 [3].

In many studies in the literature, it has been reported that disease-related variant carriers of BRCA2 in both sexes also increase the risk of some other types of cancer such as pancreatic cancer, gastric cancer, and malignant melanoma [4]. According to the universal mutation database (UMD), 3454 different variants of the BRCA2 gene have been reported until today, the clinical significance of the majority of them is still unknown [5]. One of these variants, BRCA2 c.9976A>T (K3326X), results in a termination codon at the penultimate exon, predicted to cause a truncation of the last 91 amino acids. There are conflicting publications in the literature regarding the possible effect of this variant on cancer. In this study, the importance of BRCA2 K3326X variant, detected in patients diagnosed with Turkish breast and ovarian cancer, in the etiopathogenesis of the disease, was investigated.

Method

In this retrospective study between January 2018 and January 2020, the K3326X variant was investigated in patients with breast/ovarian cancer whose genetic analysis to detect a genomic change associated with hereditary cancer was performed in Diskapi Yildirim Beyazit Training and Research Hospital Department of Medical Genetics. All of 1957 patients included in the study were older than 18 years of age and were suspected of having BRCA1/2 genes in the etiopathogenesis of their existing cancers. General demographic and clinicopathological characteristics and gene analyses results of the patients were obtained retrospectively from the patient files.

This study was conducted by considering ethical responsibilities according to the World Medical Association and the Declaration of Helsinki. A written informed consent was obtained from all participants prior to the study and this descriptive case series study was approved by the Harran University Local Ethics Committee (Approval number: HRU/21.08.14).

Peripheral blood samples were obtained from patients and Genomic DNA investigated by

the next-generation sequencing method. Analysis of the BRCA1/2 genes was performed on the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA) with the GeneRead QIAact BRCA Advanced DNA UMI Panel (Qiagen, Hilden, Germany). This panel provides 100% coverage for all exons and exon/intron junctions (up to 25 base pairs) of the BRCA1/2 genes. In addition, this panel made possible the analysis of SNVs and Indels. QIAGEN Clinical Insight (QCI™) software was used for the analysis of the data obtained. NM_007294.3 for the BRCA1 gene and NM_000059.3 for the BRCA2 gene, set as reference transcripts. In the study, the detected gene variants were classified based on the criteria in the American College of Medical Genetics and Genomics guideline.[6] In order to investigate the possible effect of the K3326X variant detected in patients with cancer on the etiopathogenesis of cancer, a control group of 432 people without cancer diagnosis were included. The control group consisted of patients with neurological, metabolic, cardiological, nephrological, ophthalmological and musculoskeletal disorders.

Sequence analysis of DNA sample obtained from the peripheral blood of these control group patients was performed using the Next Generation Sequencing (NGS) method using the Hereditary Disease Solution by Sophia Genetics (HDS_v3) on Nextseq Platform (Illumina, USA). HDS_v3 panel contained 569 genes. The raw data of the patients obtained by NGS were analyzed in a web-based bioinformatics program (https://www.sophiagenetics.com/home.html) and according to the reference genome (GRCh37 (h19)). In this group, 11 individuals were found with the K3326X variant in a heterozygous state. This group and the group with cancer patients were compared statistically in terms of the rate of having the K3326X variant.

The software "SPSS for Windows v23.0 (SPSS Inc., Chicago, IL, USA)" was used in the statistical evaluation of all results obtained. "Mean values (±) and standard deviation (SD), ±SD" were presented for scale data and percentage (%) for nominal variables Pearson chi square test. " were used in the study of the relations of the two qualitative variables. When the p-value used for the level of statistical significance was "0.05 or less", which was accepted as a meaningful result.

Results

In this study, K3326X variant was detected in 54 (2.75%) of 1957 cancer patients. Among 54 cancer patients, 52 (96.3%) carried this variant as heterozygous, two of them homozygous (3.7%). Of these 54 cancer cases, two (3.7%) were being followed up with a diagnosis of ovarian cancer and 52 (96.3%) with a diagnosis of breast cancer. The ages of diagnosis of two patients with ovarian cancer were 48 and 56, respectively, and the histopathological subtype of cancer in both patients was detected as serous adeno-carcinoma. The mean age at diagnosis of patients with breast cancer was 46.26 ± 9.32 years. Histopathological subtypes of cancer of these patients were mostly determined as invasive ductal carcinoma (IDC) (78.8%) and invasive lobular carcinoma (ILC) (11.5%). Breast cancer subtypes of the other five patients were medullary (1.9%), mucinous (IMC) (1.9%), IDC/ILC (1.9%), IDC/ILC / IMC (1.9%), IDC/IMC (1.9%). It was determined that 77.1% of the tumors of breast cancer patients were estrogen receptor (ER) positive, 73.5% progesterone receptor (PR) positive, and 61.4% had c-erbB-2 (HER2/neu) amplification. 11.1% of these patients were triple negative (TNBC). The mean age at diagnosis of TNBC patients was 48.33 ± 7.11 and the mean age at diagnosis of non-TNBC patients was 46.00 ± 9.59, and no statistically significant difference was found in the mean age at diagnosis of both groups (p = 0.568). Genomic changes of NM_007294.3 (BRCA1): c.5035delC(p.Asn 1678_ Leu1679 insTer) and NM_000059. 3(BRCA2): c.5969delA(p.Asp1990Valfs) were detected in two of the carriers heterozygously. Two patients were diagnosed with HBOC. In the other 52 patients with the K3326X variant, a

Table 1. Relationship between K3326X carrier status and cancer

K3326X	The group of patients with cancer (n=1957) n	%	The group of patients without cancer (n=432) n	%	Statistical analysis* Probability
Carrier	54	2,8	11	2,5	p=0,934
Non-carrier	1903	97,2	421	97,5	

* Chi-Square Test: Analysis of Contingency Tables

causal variant was not found in BRCA1 and BRCA2 genes. There were 432 patients in the non-cancerous group, of which 11 carried the K3326X variant. The group which included cancer patients, and other group were compared in terms of the frequency of K3326X variant carriage, and no statistically significant difference was found between the groups (p = 0.934) (Table I).

Discussion

Amino acids subjected to truncation caused by the K3326X variant in the BRCA2 gene have functional importance due to the C-terminus region where they are located. In deletions of this region, it has been observed that BRCA2 colocalization does not occur with FANCD2 protein that plays a role in the repair of DNA damage, and regulation of the checkpoint of the cycle after injury [7, 8]. Various studies investigating the cellular and biochemical effects of this variant on cancer cell lines have been reported in the literature. As a result of some studies, it has been shown that the K3326X variant has no effect on BRCA2 function, while some studies have yielded opposite results [9-11]. In the study of, Morimatsu et al; the importance of this region in mouse cells with deletion of the region containing the last 188 amino acids at the COOH terminal of the BRCA2 gene was investigated. It has been observed that the cell cycle progression rate of these cells and wild type cells are similar. However, it has been observed that cells with deletion age faster than wild ones and are more radiosensitive. As a result, it has been shown that BRCA2 deletion can accelerate cell proliferation and induce cancer through defective DNA repair.[11] These studies on BRCA2 K3326X variant have conflicting results. Sergey et al., designed an assay using mouse embryonic stem cells and bacterial artificial chromosomes to investigate the functional significance of mutations in BRCA2. In their study, they showed that the K3326X genomic modification had no effect on BRCA2 gene functions and that it was a neutral variant [9]. In another study, Wu et 190 al. investigated the functional importance and cancer relationship of various VUSs in the BRCA2 gene, and found that the K3326X variant did not cause a change in BRCA2 function at a level that would cause cancer susceptibility. In their study using three independent assays evaluating homologous recombination, cell survival, and centrosome regulation, they showed that this variant did not affect the function of the BRCA2 gene in any of the assays [10].

Various researchers have investigated the importance of this variant in cancer susceptibility in many malignancies, especially breast and ovarian cancer, and different results have been obtained. The K3326X variant, which was first described by Mazoyer in the literature, was detected at approximately 1% in both the 462 control and 513 breast cancer patients has suggested to be a polymorphic variant [12]. While the same variant was detected in the control population

by Wagner et al [13], it was found by Bergthorsson et al in a patient with breast cancer and was evaluated as a polymorphism [14]. As a result of a study conducted by Offit et al. with patients followed up for Fanconi Anemia (FA), they concluded that the BRCA2 K3326X allele could not be evaluated pathogenic [15]. Reid et al; claimed that this variant was not pathogenic, as it did not show segregation with the disease in some cancer families and its incidence in the population was the same as that of breast cancer patients [16].

In some studies, researchers claimed that the K3326X variant, considered a polymorphism, may be a risk factor. Martin et al found this variant at a higher prevalence in patients with familial pancreatic cancer than in healthy controls. The researchers have claimed that this variant has a detrimental effect and leads to an increased risk of pancreatic cancer [17]. Michailidou et al., in a meta-analysis based on GWAS (genome-wide association studies) they performed in 2013, suggested that this variant may cause a small increase in the risk of breast cancer in the individual [18]. In another GWAS conducted by the same researcher in 2015, breast cancer-related common variants located in 79 different loci were investigated and it was found that the K3326X variant could increase the risk of breast cancer by 1.26-fold [19]. In addition to these studies, we have suggested that this variant may cause an increase in the risk of some cancer types other than breast and ovarian cancer.

Howlett et al have argued that if the BRCA2 K3326X variant is compound heterozygous, it may cause FA [20]. Wang et al suggested that the BRCA2 K3326X sequence variant may have a direct effect on lung cancer development [21]. Akbari et al. detected the K3326X variant at a higher rate in 220 patients diagnosed with esophageal squamous cell carcinoma compared to healthy controls, and suggested that this variant may be a risk factor for the disease [22]. In a case diagnosed with bilateral breast cancer and melanoma, variants Q563X in the BRCA1 gene and K3326X in the BRCA2 gene were defined as double heterozygous by Palmirotta et al. It has been emphasized that the presence of a second variant with K3326X induces to have an earlier onset of the neoplasia to develop, and this variant has a strong penetrance modifier role [23]. One of the most comprehensive studies on this variant is the study by Meeks et al in 2016 involving approximately 77000 patients and 84000 controls diagnosed with breast, ovarian and prostate cancer. The researchers found a statistically significant relationship between the K3326X variant and all invasive breast cancer cases in this study, especially cases with triple-negative breast cancer and estrogen receptor negative breast cancer. In patients with lobular breast cancer, there was no significant relationship between this variant and the disease. In the same study, a strong association was observed between this variant and the disease in cases with serous ovarian cancer, but not in non-serous ovarian cancer patients. In addition, no significant association was reported between prostate cancer and K3326X. The results of this large study have suggested that the BRCA2 K3326X variant may play a role in the etiopathogenesis of breast and ovarian cancer [24,25]. In another study in the literature, the mean age at diagnosis of breast/ovarian cancer patients carrying the K3326X variant was reported as 43.2 ± 9.6 (range 28-67), and no significant difference was found in mean age at diagnosis with non-carrier patients (45.5 ± 10.6). In the same study, the researchers claimed that this variant has a different clinical significance from the BRCA2 truncating mutations located at the other 5'end, and its effect on breast and/or ovarian cancer development cannot be ignored. Therefore, they were suggested that the K3326X variant should be included in panels of all low penetration susceptibility SNPs [26].

In our study, the mean age at diagnosis of breast cancer patients was found to be 46.26 ± 9.32, similar to the literature. Although breast cancer is frequently diagnosed between the ages of 55-64 in developed countries, it has been reported that the breast cancer patient

population in Turkey is between the ages of 45-49 mostly [27].

Breast cancer is a disease that can occur with the effect of many environmental, individual and genetic risk factors, and the age of onset may vary geographically and ethnically. The age at onset of breast cancer is significantly younger in patients with BRCA1/2 pathogenic variants compared to 250 patients without BRCA1/2 [28]. For this reason, National Comprehensive Cancer Network (NCCN) guidelines include the age factor, in the testing criteria for hereditary breast and ovarian cancer [29].

Up to 70% of breast cancers are hormone-dependent, and they are usually ER-positive. TNBC is detected only in 10-20% of invasive breast cancers [30]. In some studies, it has been shown that BRCA1-associated tumors, usually TNBC and BRCA2 mutation carriers, tend to develop mostly ER-positive breast cancer. In addition, in some publications, it was reported that patients with the same causal variant of BRCA1 were diagnosed with breast cancer at a younger age compared to others [31]. In our study, no significant difference was observed between the ages of cancer diagnosis between patients with variant carriers, those with TNBC, and non-TNBCs ($p = 0.568$).

In the Genome Aggregation Database (GnomAD), it is stated that the frequency of this variant allele is approximately 1 in 151. The allelic frequency of BRCA2 K3326X variant has been reported as 1 in 845 in the African population, 1 in 251 in Ashkenazi Jewish, 1 in 379 in Latinos, 1 in 100 in Europeans, and 1 in 144 in South Asian population [32]. In this study, we investigated the frequency of BRCA2 K3326X variant carriage in cancer and non-cancerous patients who were genetically analyzed in our own laboratory, and we have found that this variant does not play an important role in the etiopathogenesis of breast/ovarian cancer.

Conclusion

We have examined the role of the K3326X variant, which has been investigated many times in the literature and whose clinical significance is not fully understood in the etiopathogenesis of the breast and ovaries in the Turkish population. Our results have provided evidence in favor that BRCA2 K3326X variant may be a benign variant. In order to determine the possible mechanism of action of this variant in cancer, comprehensive studies are needed in different populations.

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