ABSTRACT

Objective: Hereditary cancer syndromes (HCSs) are a heterogeneous group of disorders caused by germline pathogenic variations in various genes that function in cell growth and proliferation. This study aimed to describe the germline variations in patients with hereditary cancer using multigene panels.

Methods: The molecular and clinical findings of 218 patients with HCS were evaluated. In addition, 25 HCS-related genes were sequenced using a multigene panel, and variations were classified according to the American College of Medical Genetics and Genomics (ACMG) criteria. In total, 218 HCS patients predominantly with breast, colorectal, ovarian, gastric, and endometrium cancers were included.

Results: Pathogenic variations in 12 distinct genes were detected in 36 of 218 (16.5%) cases. In this study, the most affected gene was the ATM gene, in which pathogenic variations were detected in 8 of 218 cases, followed by CHEK2 (3.2%), MUTYH (3.2%), BRIP1 (1.8%), BARD1 (0.9%), TP53 (0.9%), PALB2 (0.4%), MLH1 (0.4%), MSH2 (0.4%), PM2 (0.4%), RAD50 (0.4%), and RAD51C (0.4%).

Conclusions: This study contributes to genotype-phenotype correlation in HCSs and expands the variation spectrum by introducing three novel pathogenic variations. The wide spectrum of the gene pathogenic variations detected and the presence of multiple gene defects in the same patient make the multigene panel testing a valuable tool in detecting the hereditary forms of cancer and providing effective genetic counseling and family specific screening strategies.

Keywords: Cancer predisposition, genetic counseling, hereditary cancer, next generation sequencing

ÖZ

Amaç: Herediter kanser sendromları (HCS) hücre büyümeye ve proliferasyonu üzerinde görevi yerlere saptanan genetik mutasyonlardan kaynaklanan heterojen bir grup hastalıktır. Bu çalışmada kalıtımsal kanser sendrom ön tanımla ile değerlendirilen olgulara çoklu gen paneli ile germ hatti varyasyonlarının değerlendirilmesi planlanmıştır.

Yöntemler: Kalıtımsal kanser sendromu düşünülen 218 olguna periferik kandan DNA izolasyonu sonrası 25'li gen multigen paneli kullanılarak diziendi ve varyasyonlar American College of Medical Genetics and Genomics (ACMG) kriterlerine göre değerlendirildi.

Bulgular: Meme, kolorektal, over, gastrik ve endometrium kanseri başta olmak üzere toplam 218 herediter kanser sendromu olguna değerlendirildi. Tüm çalışma grubu incelendiğinde en sık ATM gen varyasyonları (8/218, %3.6) tespit edildi ve bunu siklik sırasına göre CHEK2 (%3.2), MUTYH (%3.2), BRIP1 (%1.8), BARD1 (%0.9), TP53 (%0.9), PALB2 (%0.4), MLH1 (%0.4), MSH2 (%0.4), PMS2 (%0.4), RAD50 (%0.4), RAD51C (%0.4) varyasyonları takip etmektediydi.

Sonuçlar: Bu çalışmada farklı kanser türlerinde kalıtımsal kansere yol açan genler analiz edilmiş ve fenotip ile ilişkisinin değerlendirilmiştir. Ayrıca bu çalışmada ilk kez saptanan üç yeni varyasyon ile literatüre katkı sağlamaktadır. Patojenik varyasyon tespit edilen genlerin geniş dağılımı ve aynı hastada birden fazla genetik varyasyonun varlığı düşünüldüğünde, uygun genetik danışma ve aileye özgü tarama planlaması yapılarak çoklu gen taraması kalıtımsal kanser hastalarını değerlendirilmesinde hızlı ve etkin bir yöntem olarak görülmektedir.

Anahtar kelimeler: Kanser yatkınlığı, genetik danışma, herediter kanser, yeni nesil dizileme
INTRODUCTION

Cancer is a multifactorial disease associated with various genes involved in multiple cellular functions such as cell cycle regulation, DNA repair systems, and apoptosis. Germline molecular variations in these genes result in cancer predisposition in humans, defined as hereditary cancer syndromes (HCSs). HCSs are characterized by early-onset cancer cases occurring in the same family. Germline pathogenic variations carriers of HCS-related genes constitute 5%-10% of all cancer cases. Describing the underlying molecular defects in these patients is valuable in terms of the clinical approach for cancer patients and screening and follow-up strategies for asymptomatic family members.

Although some clinical findings such as hyperpigmented macules around the lips and oral mucosa are specific to Peutz Jeghers syndrome (PJS), clinical and genetic heterogeneity in HCSs make molecular diagnosis difficult. Screening genes singly is time consuming and not cost effective. Next, generation sequencing technology allows us to screen multiple genes efficiently in a short time. In clinical practice, multigene panel testing has advantages for screening patients according to tumor characteristics, accompanying features, and family history.

Breast cancer is the most common cancer and the leading cause of cancer-related deaths in women worldwide. Pathogenic BRCA1 and BRCA2 variations account for 20% of familial breast cancers, and cases carrying a pathogenic variation in one of these genes have 40%-80% lifetime breast cancer risk. In BRCA-negative cases, older related breast cancer are screened.

In colorectal cancer (CRC) and endometrial cancer, histochemical evaluation of tumor tissue may predict the underlying molecular defect. For instance, microsatellite instability (MSI) and expression loss of mismatch repair (MMR) genes may lead to the evaluation of Lynch syndrome (LS)-related genes. Another CRC type, colon familial adenomatous polyposis (FAP), characterized by multiple polyps is associated with APC and MUTYH variations, which are easily predictable as FAP or MUTYH-Associated Polyposis (MAP) after clinical evaluation, respectively.

HCSs with predictable underlying genetic defects may be evaluated using specific targeted sequencing panels. Panels including more genes associated with HCSs are required for patients with nonspecific clinical features that may be related to various genes or absence of enough data for prediction. This approach prevents the difficulties in the interpretation of variants of uncertain significance; however, it may be time consuming in clinical practice.

In this study, in 218 without a specific HCS (such as PJS, FAP etc.) diagnosis were evaluated via a multigene panel testing including 25 HCS-causing genes, and the clinical outcomes of patients having pathogenic or likely pathogenic variations were discussed.

MATERIALS and METHODS

Case Selection

A total of 218 patients who were evaluated in our outpatient clinic with a diagnosis of HCS between 2016 and 2020 were selected for this study. As selection criteria, patients who developed two types of cancer or having a family history (≥2 cases in 1st, 2nd, or 3rd degree relatives, at the same side of pedigree) or early-onset (compared to related cancer median age) of cancer were included in the study. All patients having breast or ovarian cancer were screened for BRCA1 and BRCA2 genes, and patients with a molecular diagnosis of BRCA-related hereditary breast ovarian cancer syndrome were excluded. Patients with CRC who were not considered as LS according to molecular findings in tumor tissue, such as absence of MSI and expression loss of mismatch repair genes and FAP/MAP according to colonoscopy findings, were included in the study. However, the tumor molecular evaluation results of 6 patients with CRC were not available. Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images. The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (protocol no: 09.2020.751, date: 24.07.2020). All patients received pre- and post-test counseling from a medical genetics specialist, and informed consents were obtained via face-to-face interviews. The patients were evaluated in the outpatient clinic in terms of cancer type, age of onset, and family history.

Molecular Analysis

We sequenced 25 genes associated with HCSs (ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FAM175A, MRE11A, NBN, PALB2, PIK3CA, RAD50, RAD51C, RAD51D, TP53, XRCC2, MLH1, MSH2, MSH6, PMS2, APC, MUTYH, PTEN, and STK11) in the Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA) using the HCS_v1 kit (SOPHiA Genetics, Boston USA) after DNA isolation from peripheral blood samples taken with an EDTA tube and obtaining informed consent from the patients. The data obtained were analyzed in Sophia DDM analysis program. To compare variants, sequencing data was aligned to human reference genome, hg19. Regarding the confirmation and segregation analysis of the
detected variants, the target region was replicated with the designed primers and then sequenced with the ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific, MA USA) device by the Sanger sequencing method. The Human Gene Mutation Database Professional (HGMD, 2020) and ClinVar databases were screened for the known variations, and novel variants detected in the study were evaluated according to American College of Medical Genetics and Genomics criteria.

Statistical Analysis

Data were evaluated via Microsoft Excel for Mac (version 15.33) application. Mean ages and percentage values were obtained using this software.

RESULTS

Out of 218 cases, 174 female and 44 male cases suspected for HCS were screened for variations via a targeted panel including 25 genes. The patients were aged between 18 and 75 years, and the median age was 44. Among the 218 cases, 131 (60%) were breast cancer patients, of which 8 had contralateral breast cancer and 13 developed a second type of cancer in their follow-up. Only one male breast cancer patient was present in the study group. Colon cancer was present in 21 patients, accounting of 9.6% of all cases, and one of them had leukemia. It is followed by ovarian (n=16) and gastric cancer (n=11), which accounted for 7% and 5% of all patients. In total, 16 patients (7.3%) had a history of two distinct types of cancer at the time of study. In addition, 213 (98%) patients had at least two relatives diagnosed with cancer, and 5 (2%) patients had no family history of cancer but were included in the study because they had two malignancies co-occurring or were diagnosed at a very early age, which lead to the suspicion of HCS. The characteristics of the study population are shown in Table 1.

**Table 1. Characteristics of patients.**

| Cancer type       | Number of patients (%) | Sex (F/M) | Age range/median age | No family history | 1st degree | 2nd degree | 3rd degree | ≥4th degree |
|-------------------|------------------------|-----------|----------------------|-------------------|------------|------------|------------|-------------|
| Breast Ca         | 118 (54%)              | 117/1     | 27-79/44             | 83 (70.3%)        | 23 (19.5%) | 10 (8.5%)  | 2 (1.7%)   |
| + Endometrium Ca* | 3                      | 3/0       |                      | 1                  | 1          | 1          | 1          |
| + Lung Ca*        | 2                      | 1/0       |                      | 1                  | 1          | 1          | 1          |
| + Ovarian Ca*     | 2                      | 2/0       |                      | 2                  |            |            |            |
| + Skin Ca*        | 2                      | 2/0       |                      | 1                  | 1          |            |            |
| + CRC*            | 1                      | 1/0       |                      | 1                  |            |            |            |
| + Renal Ca*       | 1                      | 1/0       |                      | 1                  |            |            |            |
| + Bladder Ca*     | 1                      | 1/0       |                      | 1                  |            |            |            |
| + Lymphoma*       | 1                      | 1/0       |                      | 1                  |            |            |            |
| Colorectal Ca     | 20 (9%)                | 9/11      | 19-74/45             | 11                 | 8          | 1          |            |
| + Leukemia*       | 1                      | 0/1       |                      | 1                  |            |            |            |
| Ovarian Ca        | 16 (7.3%)              | 16/0      | 28-60/38             | 10                 | 5          | 1          |            |
| Gastric Ca        | 11 (5%)                | 2/9       | 18-71/41             | 2                  | 5          | 4          |            |
| Pancreas Ca       | 8 (3.6%)               | 2/6       | 31-72/55             | 4                  | 4          |            |            |
| + CRC*            | 1                      | 0/1       |                      | 1                  |            |            |            |
| Endometrium Ca    | 8 (3.6%)               | 8/0       | 42-70/52             | 7                  | 1          |            |            |
| Prostat Ca        | 8 (3.6%)               | 0/8       | 46-75/64             | 4                  | 4          |            |            |
| Lung Ca           | 7 (3.2%)               | 1/6       | 50-75/67             | 3                  | 4          |            |            |
| Thyroid Ca        | 2 (1%)                 | 2/0       | 30,34                | 2                  |            |            |            |
| Bladder Ca        | 2 (1%)                 | 1/1       | 37,50                | 2                  |            |            |            |
| Nasopharynx Ca    | 1 (0.5%)               | 1/0       | 51                   | 1                  |            |            |            |
| Brain Ca          | 1 (0.5%)               | 1/0       | 41                   | 1                  |            |            |            |
| Renal Ca + Leukemia | 1 (0.5%)             | 1/0       | 39                   | 2 (0.9%)           |            |            |            |

*Sign “*” expresses the second cancer types developed in addition to the cancer type stated upper line, in order of diagnosis. Ca: Cancer, F: Female, M: Male, CRC: Colorectal cancer
Pathogenic variations in 12 distinct genes were detected in 36 of 218 (16.5%) cases. In the whole study group, the most identified findings were ATM alterations, which were detected in 8 of 218 (3.6%), patients, followed by CHEK2 (3.2%), MUTYH (3.2%), BRIP1 (1.8%), BARD1 (0.9%), TP53 (0.9%), PALB2 (0.4%), MLH1 (0.4%), MSH2 (0.4%), PMS2 (0.4%), RAD50 (0.4%), and RAD51C (0.4%). The distribution of the frequency of patients with pathogenic/likely pathogenic variations according to the genes are shown in Figure 1.

Among 118 isolated BRCA-negative breast cancer patients, of which eight had bilateral breast cancer, 18 (15.2%), including two patients with bilateral breast cancer, were found to have a deleterious variation in one of the HCS-related genes, and all were female. In the ATM gene 2 frameshift, a nonsense and a missense variation were detected in 4 patients. One of the frameshift variations was novel (c.6840_6844delGTACA). Four patients were carrying pathogenic CHEK2 variations, of which one was a novel variation (c.1094_1095+4del), causing 6 nucleotide deletions including the last two nucleotides of exon 9 and four nucleotides in intron 9. It was predicted to cause a shift in the reading frame and to stop the translation at the following third codon. In three patients with breast cancer, three previously reported pathogenic variations in MUTYH were detected. One of the patient was homozygous for c.884C>T (p.Pro295Leu) variation, which is a known cause of MAP. In this patient, breast cancer was diagnosed at the age of 54; however, colonoscopy was not performed to date due to the absence of complaints. In the BRIP1 gene, three nonsense variations were detected in three breast cancer patients. In two cases, a novel splice site variation (c.1903+2T>A) and a known missense variation were found in the BARD1 gene. The other genes affected in patients with breast cancer were TP53 (n=1) and RAD51C (n=1).

In 5 of 20 (25%) isolated patients with colon cancer, pathogenic variations were detected in the ATM, CHEK2, BRIP1, MLH1, and MSH2 genes, which were all previously reported pathogenic variations.

Pathogenic variations were detected in three of seven gastric cancer patients, which accounts 27%. Two of the variations were missense variations in the MUTYH gene, and a splice site variation was detected in the PALB2 gene. The variations and cancer type of the pathogenic variation carriers are listed in Table 2. Four (25%) of 16 patients having two distinct cancer types were found to have deleterious variations.

Figure 1. Distributions of detected pathogenic/likely pathogenic variations in HCSs patients.
HCS: Hereditary cancer syndrome
Table 2. Detected pathogenic/likely pathogenic variations in HCS patients.

| Case | Sex | Age | Clinical features | Family history | Gene | Variation | Coding consequence | Variation status |
|------|-----|-----|-------------------|----------------|------|-----------|-------------------|-----------------|
| 1    | F   | 37  | Breast Ca         | +              | ATM  | c.5443delG p.Asp1815Thrfs*13 | Frameshift       | RP              |
| 2    | F   | 68  | Breast Ca         |                | ATM  | c.5644C>T (p.Arg1882*)        | Nonsense         | RP              |
| 3    | F   | 29  | Breast Ca         | +              | ATM  | c.6154G>A (p.Glu2052Lys)      | Missense         | RP              |
| 4    | F   | 60  | Breast Ca         | +              | ATM  | c.6840_6844delGTACA (p.Tyr2281Phefs*3) | Frameshift | N               |
| 5    | F   | 45  | Breast Ca         | +              | CHEK2| c.793-1G>A                          | Splice site      | RP              |
| 6    | F   | 41  | Breast (bilateral)| +              | CHEK2| c.1063delC (p.Leu355Cysfs*10)   | Frameshift       | RP              |
| 7    | F   | 36  | Breast Ca         | +              | CHEK2| c.1094_1095+4del (Lys365Asnfs*3) | Frameshift       | N               |
| 8    | F   | 54  | Breast Ca         | +              | MUTYH| c.884C>T (p.Pro295Leu) HOM     | Missense         | RP              |
| 9    | F   |     | Breast Ca         | +              | MUTYH| c.884C>T (p.Pro295Leu)         | Missense         | RP              |
| 10   | F   | 45  | Breast Ca         | +              | BRI1 | c.484C>T (p.Arg162*)           | Nonsense         | RP              |
| 11   | F   | 51  | Breast Ca         |                | BRI1 | c.2392C>T (p.Arg798*)          | Nonsense         | RP              |
| 12   | F   | 36  | Breast Ca         | -              | BRI1 | c.1776G>A p.Trp592*            | Nonsense         | RP              |
| 13   | F   | 42  | Breast Ca         | +              | BARD1| c.1903+2T>A                    | Splice site      | N               |
| 14   | F   | 52  | Breast Ca         | +              | BARD1| c.1409A>G (p.Asn470Ser)        | Missense         | RP              |
| 15   | F   | 27  | (Bilateral)       | +              | TP53 | c.445dupT (p.Ser149Phefs*32)   | Frameshift       | RP              |
| 16   | F   | 38  | Breast Ca         | +              | TP53 | c.437G>A p.Trp146*             | Nonsense         | RP              |
| 17   | F   | 44  | Breast Ca         | +              | RAD51C| c.904+5G>T                    | In frame deletion| RP              |
| 18   | F   | 60  | Breast Ca + Endometrium Ca | - | CHEK2 | c.599T>C p.Ile200Thr | Missense         | RP              |
| 19   | F   | 46  | Breast Ca + Lymphoma | + | ATM  | c.7327C>T (p.Arg2443*)         | Nonsense         | RP              |
| 20   | F   | 26  | CRC                | +              | ATM  | c.6047A>G (p.Asp2016Gly)       | Missense         | RP              |
| 21   | F   | 30  | CRC                | +              | CHEK2| c.678G>C p.Leu226Phe           | Missense         | RP              |
| 22   | F   | 55  | CRC                | +              | BRI1 | c.139C>G p.Pro47Ala            | Missense         | RP              |
| 23   | M   | 73  | CRC                | +              | MLH1 | c.588+3_588+6del               | Splice site      | RP              |
| 24   | M   | 26  | CRC                | +              | MSH2 | c.942+3A>T                     | Splice site      | RP              |
| 25   | M   | 19  | CRC + Leukemia     | +              | MLH1 | c.2115C>T (p.Gln719*) hom      | Nonsense         | RP              |
| 26   | M   | 47  | Pancreas + CRC     | -              | ATM  | c.7088delA p.Lys2363Argfs*3    | Frameshift       | RP              |
| 27   | M   | 36  | Pancreas Ca        | +              | ATM  | c.2125-1G>A                    | Splice site      | RP              |
| 28   | M   | 18  | Gastric Ca         | -              | MUTYH| c.1145G>A (p.Gly382Asp)        | Missense         | RP              |
| 29   | M   | 41  | Gastric Ca         | +              | MUTYH| c.884C>T (p.Pro295Leu)         | Missense         | RP              |
| 30   | M   | 42  | Gastric Ca         | +              | PALB2| c.2587-1G>C                     | Splice site      | RP              |
| 31   | M   | 45  | Eusophagus Ca      | +              | BRCA1| c.3794delA (p.Asn1265Ilefs*3)  | Frameshift       | RP              |
| 32   | M   | 28  | Ovarian Ca         | +              | RAD50| c.2083C>T (p.Gln695*)          | Nonsense         | RP              |
| 33   | M   | 46  | Prostate Ca        | +              | CHEK2| c.678G>C p.Leu226Phe           | Missense         | RP              |
| 34   | M   | 30  | Thyroid Papillary Ca | + | CHEK2 | c.499G>A (p.Gly167Arg)        | Missense         | RP              |

RP: Reported previously, N: Novel, Ca: Cancer, F: Female, M: Male, CRC: Colorectal cancer, HCS: Hereditary cancer syndrome
The pathogenic variation detection rate according to phenotypes and affected genes are summarized in Table 3. A 46-year-old female patient with a diagnosis of synchronous B-cell lymphoma and breast cancer had a nonsense variation in the ATM gene and a missense variation in the MUTYH gene. This was the only patient having known pathogenic variations in two distinct HCS-related genes. Her parents were 82 and 84 years old and had no history of malignancy. She had a sister who died at the age of 48 due to endometrial cancer, and five healthy brothers aged between 43 and 60. She also had three cousins who died due to CRC in their 40s.

**DISCUSSION**

Multigene panel testing is a rapid method of identifying the underlying molecular defects in genetically heterogenous diseases. However, the results may be confusing due to variations of uncertain significance and difficulties in interpreting novel variations. Additionally, since the test results concern the whole family, the decision to undergo testing and management of at-risk family members may be challenging in the absence of a clear segregation data due to test rejection, absence of living cancer patients in the family, or incomplete penetrance.

The pathogenic variation detection rate was 16.5% regardless of the cancer type. LaDuca et al.\(^{11}\) reported 8.3% positive results in a study including 2,079 HCS patients via multigene panel testing. In a cohort of BRCA1/2 negative high-risk patients including 122 patients, the pathogenic variation detection rate was reported as 11%\(^{12}\). The differences in pathogenic variation detection rate may be due to the criteria used for study inclusion, the ethnicities, the genes included in the panel, and the evaluation of gene deletions and copy number variations (CNVs). In our study, family history and multiple cancer development were the main criteria for HCS panel testing. Only two patients with gastric cancer who did not meet these criteria were included in the study because of their early age of diagnosis (18 and 24). Interestingly, Samadder et al.\(^{13}\) reported a study indicating the inadequacy of phenotype or family history-based testing criteria in detecting the pathogenic variations in cancer patients, in which approximately 48% of the patients carrying germline pathogenic variants would not have been detected using standard guidelines. In this study, the pathogenic variation detection rate was reported as 13.3% in 282 moderate- and high-penetrance cancer susceptibility genes.

**Table 3. Phenotypes of patients having pathogenic/likely pathogenic variations.**

| Patients (Nr of cases in study population) % | Cancer type | P/LP variation carrying patients/total patients (%) | Effected genes |
|--------------------------------------------|-------------|-----------------------------------------------|----------------|
| One cancer type (n=202) 92.7%              | Breast Ca   | 19/118 (16%)                                  | ATM, CHEK2, MUTYH, BRIP1, BARD1, TP53, RAD51C, CHEK2, TP53 |
|                                            | · Bilateral breast Ca | 2/8 (25%)                                   |                |
|                                            | CRC         | 5/20 (25%)                                   | ATM, CHEK2, BRIP1, MLH1, MSH2 |
|                                            | Ovarian Ca  | 1/16 (6%)                                    | RAD50          |
|                                            | Gastric Ca  | 3/11 (27%)                                   | MUTYH, PALB2   |
|                                            | Pancreas Ca | 1/8 (12.5%)                                  | ATM            |
|                                            | Prostat Ca  | 1/8 (12.5%)                                  | CHEK2          |
|                                            | Lung Ca     | 1/7 (14%)                                    | MUTYH          |
|                                            | Thyroid Ca  | 1/2 (50%)                                    | CHEK2          |
| Two cancer types (n=16) 7.3%              | Breast + Endometrium Ca | 1/3                                          | CHEK2          |
|                                            | Breast Ca + Lymphoma | 1/1                                          | ATM and MUTYH  |
|                                            | CRC + Leukemia | 1/1                                         | PMS2           |
|                                            | Pancreas Ca + CRC | 1/1                                         | ATM            |
| Total                                      | 36/218 (16.5%)                                   |                |

Ca: Cancer, CRC: Colorectal cancer, P: Pathogenic, LP: Likely pathogenic
to our study, which was detected 3.2% of cases and was the second most common cause of HCS in our study group. Yadav et al. reported pathogenic ATM variation in two of 122 BRCA-negative patients, which accounted for 1.6% of the tested population, and pathogenic CHEK2 variation was detected in four patients, accounting for 3.2% of the study population. ATM and CHEK2 are known as moderate penetrance genes, which result in a two to four-fold increase in breast cancer risk and are also associated with other cancer types such as pancreas, colon, prostate cancer, and melanoma.

*MUTYH* was another commonly affected gene in our study. We detected known deleterious MUTYH variations in seven patients, accounting for 3.2% of the study group and 17% of the pathogenic variation positive patients. The frequency of pathogenic MUTYH variation was significantly high in our study, and recurrent c.884C>T (p.Pro295Leu) variation was detected in 4 of 7 patients, which we previously detected as the most common variation in the MUTYH gene in Turkish MAP (unpublished data). In five patients, pathogenic MUTYH variation was detected in the heterozygous state. Although monoallelic MUTYH variations were excluded from calculations in some of the studies, it is known that heterozygous pathogenic MUTYH variation carriers have increased risk of colorectal, gastric, endometrial, and liver cancers.

Interestingly, in one 54-year-old patient with breast cancer, we detected a homozygous c.884C>T (p.Pro295Leu) variation, which is a known recurrent variation in MAP cases. However, our case presented no complaints, indicating that MAP and colonoscopy had not been performed previously.

As the most common cause, BRCA1 and BRCA2 pathogenic variations are responsible for 20% of hereditary breast and ovarian cancer. Therefore, in our center, we initially test all breast and ovarian cancer patients for single nucleotide variations and CNVs in BRCA1 and BRCA2 variations. All breast and ovarian cancer patients in this study were BRCA-negative patients proven via sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis of BRCA1 and BRCA2 genes. In isolated breast cancer patients, we detected a pathogenic variation rate of 15.2%. The affected genes were *ATM*, *CHEK2*, *MUTYH*, *BRIP1*, *BARD1*, *TP53*, and *RAD51C* in order of variation detection rate. Ovarian cancer patients accounted for 7.3% (n=16) of our cohort, and a nonsense variant (c.2083C>T; p.Gln695*) was detected in only one patient in the RAD50 gene. In a study including 20,590 breast cancer patients, pathogenic variations were found to be predominately in the CHEK2, MUTYH, ATM, and PALB2 genes, similar to our study.

The presence of bilateral breast cancer is an important indication for germline variation screening. In a study including 5,589 breast cancer patients, bilateral breast cancer frequency was 11.3%, and deleterious variations were detected in 8.3% of this group. In our study group, 8 of 118 (6%) breast cancer patients had synchronous or metachronous breast cancer, and deleterious variations in the CHEK2 and TP53 genes were detected in two of them.

Two main genetic syndromes related to hereditary CRC are defined. Polyposis syndromes, characterized by multiple polyps (10s-100s) in the colon and associated with *APC* and *MUTYH* genes predominantly. Hereditary nonpolyposis CRC, resulting from MMR gene defects, is the most common cause of hereditary CRCs. Therefore, colon cancer patients are screened via the LS panel, including the *MSH2*, *MSH6*, *MLH1*, and *PMS2* genes initially, if there is evidence of mismatch repair defect in the tumor tissue evaluation. In the 20 remaining patients with isolated CRC and no data about the tumor expression status, five (25%) had pathogenic variations in the *ATM*, *CHEK2*, *BRIP1*, *MLH1*, and *MSH2* genes. Erdem and Bahsi reported that 13 of 162 (8%) CRC patients had pathogenic and likely pathogenic variations in the *ATM*, *BRCA2*, *CHEK2*, *MLH1*, *MSH2*, *MUTYH*, *PMS2*, *RINT1*, and *TP53* genes. The pathogenic variation detection rate seemed higher than expected; however, our CRC population was small to make a conclusion.

Among 11 gastric cancer (GC) patients, 3 had a pathogenic variation, indicating the highest pathogenic variation detection rate (27%) according to cancer type in this study. Deleterious MUTYH variations and pathogenic PALB2 variation were detected in 2 patients and 1 patient, respectively. Although pathogenic CDH1 variations are the most common cause of hereditary GCs, none of the GC patients had pathogenic CDH1 variation in this study. Previous studies reported that MUTYH is related to GC, and it is not reported as the most common cause of GC; however, in this study, two of three pathogenic variation positive GC patients were found to be carriers for a pathogenic MUTYH variation

Pathogenic variations were detected in 4 of 16 (25%) patients having two distinct cancer types. Two of them had breast cancer, accompanied by B-cell lymphoma in one case, with germline pathogenic variations in the *ATM* and *MUTYH* genes. In the other case, endometrial cancer was present as the second malignancy, and a pathogenic CHEK2 variation was detected. In a recent study including 2,657 patients, the incidence of metachronous cancers among breast cancer patients were reported as 4.1%, in which endometrial cancer accounted for 9.3%
and lymphoma for 0.9%23. Germline pathogenic CHEK2 variations are well-known breast cancer predisposing defects and are also related with an increased risk for endometrial cancer24. A homozygous nonsense PMS2 variation was detected in a 19-year-old male having CRC and leukemia. PMS2 is a MMR gene associated with constitutional mismatch repair deficiency syndrome (CMMRDS). CMMRDS is an autosomal recessive disorder characterized by childhood brain tumors, hematological malignancies, and gastrointestinal cancer in the second and third decades of life25. Since parents of the patient are obligate carriers, they should be evaluated for LS, which result from PMS2 heterozygous variations.

We detected three novel likely pathogenic variations in three patients. The first was a five-nucleotide deletion variation (c.6840_6844delGTACA) in the ATM gene in a 60-year-old breast cancer patient, which is predicted to cause a shift in reading frame and truncated protein. The second novel variation causing 6 nucleotide deletions (c.1094_1095+4del; Lys365Asnfs*3) extending from the last two nucleotides of exon 9 to the first four nucleotides of intron 9 was detected in a 36-year-old breast cancer patient. The last novel variation c.1903+2T>A, disrupting donor splice site of exon 9 in the BARD1 gene, was detected in a 42-year-old breast cancer patient.

This study is performed in a single center, experienced predominantly in breast and CRCs. Therefore, BC and CRC patients constitute most of the study group, resulting in a less heterogeneous cancer population. Previous studies in the Turkish population were commonly based on a certain type of cancer; however, in a study including BC and CRC cases, the pathogenic variation detection rate was reported as 17.2% and 26.4%, respectively26. These data are similar to our data, reported as 16% and 28.5% in this study, respectively (Table 3).

Although the study group comprised patients from distinct regions of Turkey, it does not represent the entire Turkish population.

**CONCLUSION**

To our knowledge, this is the first report evaluating clinical and molecular features of hereditary cancer patients regardless of malignancy type in the Turkish population. High rate of pathogenic ATM variations is a striking result, which is also important in populations having high consanguineous marriage rates such as Turkey. This study also contributes to genotype–phenotype correlation in HCSs and expands the variation spectrum, introducing three novel pathogenic variations. The wide spectrum of gene variations detected and presence of multiple gene defects in the same patient make the multigene panel testing a valuable method of detecting the hereditary forms of cancer and providing effective genetic counseling and family specific screening strategies.

**Ethics**

**Ethics Committee Approval:** The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (protocol no: 09.2020.751, date: 24.07.2020).

**Informed Consent:** All patients received pre- and post-test counseling from a medical genetics specialist, and informed consents were obtained via face-to-face interviews.

**Peer-review:** Externally and internally peer-reviewed.

**Author Contributions**

Surgical and Medical Practices: E.A.A., A.T., C.A., A.I.G., Concept: E.A.A., A.I.G., Design: E.A.A., Data Collection and/or Processing: E.A.A., A.T., C.A., Analysis and/or Interpretation: E.A.A., A.T., C.A., O.Y., A.I.G., Literature Search: E.A.A., Writing: E.A.A.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**REFERENCES**

1. Garber JE, Offit K. Hereditary cancer predisposition syndromes. J Clin Oncol. 2005;23:726-92.
2. Wu M, Krishnamurthy K. Peutz-Jeghers Syndrome. [Updated 2021 Jul 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535357/.
3. Kwong A, Shin VY, Ho JC, et al. Comprehensive spectrum of BRCA1 and BRCA2 deleterious mutations in breast cancer in Asian countries. J Med Genet. 2016;53:15-23.
4. Fackenthal JD, Olopade OI. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat Rev Cancer. 2007;7:937-48.
5. Bonnet D, Selves J, Toulas C, et al. Simplified identification of Lynch syndrome: a prospective, multicenter study. Dig Liver Dis. 2012;44:515-22.
6. Kinzler KW, Nilbert MC, Su LK, et al. Identification of FAP locus genes from chromosome 5q21. Science. 1991;253:661-5.
7. Al-Tassan N, Chmiel NH, Maynard J, et al. Inherited variants of MYH associated with somatic G:C--> T:A mutations in colorectal tumors. Nat Genet. 2002;30:227-32.
8. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: 2008 update. Genome Med. 2009;1:13.
9. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018;46:D1062-7.

10. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19:4-23.

11. LaDuca H, Stuenkel AJ, Dolinsky JS, et al. Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. Genet Med. 2014;16:830-7.

12. Yadav S, Reeves A, Campian S, Paine A, Zakalik D. Outcomes of retesting BRCA negative patients using multigene panels. Fam Cancer. 2017;16:319-28.

13. Samadder NJ, Riegert-Johnson D, Boardman L, et al. Comparison of Universal Genetic Testing vs Guideline-Directed Targeted Testing for Patients With Hereditary Cancer Syndrome. JAMA Oncol. 2021;7:230-7.

14. Tsoulosis GN, Papadopoulou E, Apessos A, et al. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. BMC Cancer. 2019;19:535.

15. Choi M, Kipps T, Kurzrock R. ATM Mutations in Cancer: Therapeutic Implications. Mol Cancer Ther. 2016;15:1781-91.

16. Apostolou P, Papasotiropoulos I. Current perspectives on CHEK2 mutations in breast cancer. Breast Cancer (Dove Med Press). 2017;9:331-5.

17. Win AK, Cleary SP, Dowty JC, et al. Cancer risks for monoallelic MUTYH mutation carriers with a family history of colorectal cancer. Int J Cancer. 2011;129:2256-62.

18. O’Leary E, Iacoboni D, Holle J, et al. Expanded Gene Panel Use for Women With Breast Cancer: Identification and Intervention Beyond Breast Cancer Risk. Ann Surg Oncol. 2017;24:3060-6.

19. Hauke J, Horvath J, Groß E, et al. Gene panel testing of 5589 BRCA1/2-negative index patients with breast cancer in a routine diagnostic setting: results of the German Consortium for Hereditary Breast and Ovarian Cancer. Cancer Med. 2018;7:1349-58.

20. Erdem HB, Bahsi T. Spectrum of germline cancer susceptibility gene mutations in Turkish colorectal cancer patients: a single center study. Turk J Med Sci. 2020;50:1015-21.

21. Fitzgerald RC, Hardwick R, Huntsman D, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. 2010;47:436-44.

22. Zhou J, Zhao Z, Zhang Y, et al. Pathogenic Germline Mutations in Chinese Patients with Gastric Cancer Identified by Next-Generation Sequencing Oncology. 2020;98:583-8.

23. Kim JY, Song HS. Metachronous double primary cancer after treatment of breast cancer. Cancer Res Treat. 2015;47:64-71.

24. Stolarova L, Kleiblova P, Janatova M, et al. CHEK2 Germline Variants in Cancer Predisposition: Stalemate Rather than Checkmate. Cells. 2020;9:2675.

25. Ripperger T, Schlegelberger B. Acute lymphoblastic leukemia and lymphoma in the context of constitutional mismatch repair deficiency syndrome. Eur J Med Genet. 2016;59:133-42.

26. Akcay IM, Celik E, Agaoglu NB, et al. Germline pathogenic variant spectrum in 25 cancer susceptibility genes in Turkish breast and colorectal cancer patients and elderly controls. Int J Cancer. 2021;148:285-95.