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Original Article

Multigene Panel Testing in Turkish Hereditary Cancer Syndrome Patients

Türk Toplumunda Kalıtsal Kanser Sendromu Hastalarında Çoklu Gen Panel Taraması

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ABSTRACT

Objective: Hereditary cancer syndromes (HCSs) are a heterogenous 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%), PMS2 (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 büyümesi ve proliferasyonunda görevli genlerde saptanan germline mutasyonlardan kaynaklanan heterojen bir grup hastalıktır. Bu çalışmada kalıtımsal kanser sendrom ön tanısıyla değerlendirilen olgularda çoklu gen paneli ile germ hattı varyasyonlarının değerlendirilmesi planlanmıştır.

Yöntemler: Kalıtımsal kanser sendromu düşünülen 218 olgudan periferik kandan DNA izolasyonu sonrası HCS ile ilişkili 25 gen multigen panel kullanılarak dizilendi ve varyasyonlar American College of Medical Genetics and Genomics (ACMG) kriterlerine göre değerlendirildi.

Bulgular: Meme, kolorektal, over, gastrik ve endometriyum kanseri başta olmak üzere toplam 218 herediter kanser sendromlu olgu değerlendirildi. Tüm çalışma grubu incelendiğinde en sık ATM gen varyasyonları (8/218, %3,6) tespit edildi ve bunu sıklık 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 etmekteydi.

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

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

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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 ocurring in the same family. Germline pathogenic variations carriers of HCS-related genes constitute 5%-10% of all cancer cases[1]. 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 heterogenity in HCSs make molecular diagnosis difficult[2]. 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[3]. 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[4]. In BRCA-negative cases, rarer genes related to 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 in missmatch repair (MMR) genes may lead to the evaluation of Lynch syndrome (LS)-related genes[5]. 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[6,7].

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 evalutation 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 Ilumina 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[8-10].

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 betweeen 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 history in relatives [closest cancer (+) relative]			
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		
+ Lung Ca*	2	1/0			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		1			
Total	218	174/44	18-75/44	5 (2.3%)	142 (65.1%)	57 (26.2%)	12 (5.5%)	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 missene 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.

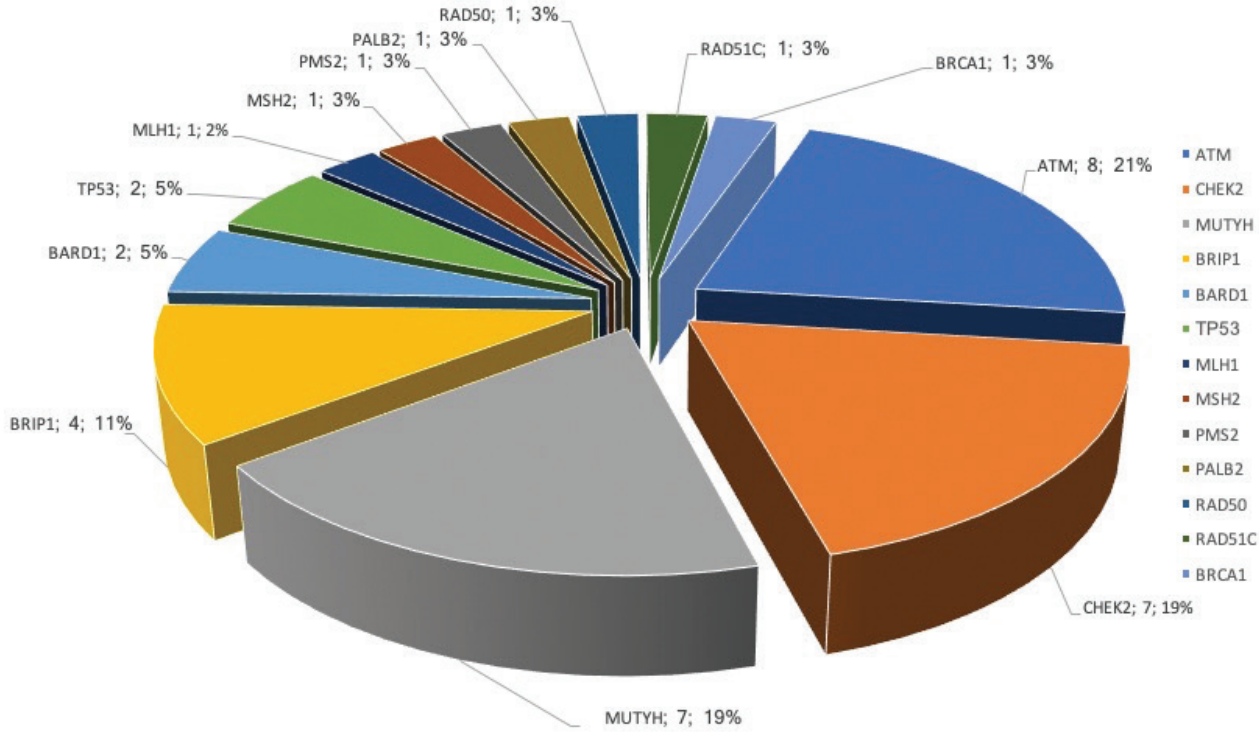


Figure 1. Distributions of detected pathogenic/likely pathogenic variations in HCSs patients.

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 Ca (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	+	*MUTYH*	c.1437_1439del (p.(Glu480del)	In frame deletion	RP
11	F	45	Breast ca	+	*BRIP1*	c.484C>T p.(Arg162*)	Nonsense	RP
12	F	51	Breast Ca	+	*BRIP1*	c.2392C>T p.(Arg798*)	Nonsense	RP
13	F	36	Breast Ca	-	*BRIP1*	c.1776G>A p.(Trp592*)	Nonsense	RP
14	F	42	Breast Ca	+	*BARD1*	c.1903+2T>A	Splice site	N
15	F	52	Breast Ca	+	*BARD1*	c.1409A>G (p.Asn470Ser)	Missense	RP
16	F	27	Breast Ca (Bilateral)	+	*TP53*	c.445dupT p.(Ser149Phefs*32)	Frameshift	RP
17	F	38	Breast Ca	+	*TP53*	c.437G>A p.(Trp146*)	Nonsense	RP
18	F	44	Breast Ca	+	*RAD51C*	c.904+5G>T	Intronic	RP
19	F	60	Breast Ca + Endometrium Ca	-	*CHEK2*	c.599T>C p.Ile200Thr	Missense	RP
20	F	46	Breast Ca + Lymphoma	+	*ATM*	c.7327C>T p.(Arg2443*)	Nonsense	RP
					MUTYH	c.884C>T (p.Pro295Leu)	Missense	RP
21	F	26	CRC	+	*ATM*	c.6047A>G (p.Asp2016Gly)	Missense	RP
22	F	30	CRC	+	*CHEK2*	c.678G>C p.Leu226Phe	Missense	RP
23	F	55	CRC	+	*BRIP1*	c.139C>G p.Pro47Ala	Missense	RP
24	M	73	CRC	+	*MLH1*	c.588+3_588+6del	Splice site	RP
25	F	26	CRC	+	*MSH2*	c.942+3A>T	Splice site	RP
26	M	19	CRC + Leukemia	+	*PMS2*	c.2155C>T p.(Gln719*) hom	Nonsense	RP
27	M	47	Pancreas + CRC	-	*ATM*	c.7088delA p.Lys2363Argfs*3	Frameshift	RP
28	M	36	Pancreas Ca	+	*ATM*	c.2125-1G>A	Splice site	RP
29	M	18	Gastric Ca	-	*MUTYH*	c.1145G>A (p.Gly382Asp)	Missense	RP
30	M	41	Gastric Ca	+	*MUTYH*	c.884C>T p.(Pro295Leu)	Missense	RP
31	M	42	Gastric Ca	+	*PALB2*	c.2587-1G>C	Splice site	RP
32	M	45	Eusophagus Ca	+	*BRCA1*	c.3794delA (p.Asn1265Ilefs*3)	Frameshift	RP
33	7019	65	Lung Ca	-	*MUTYH*	c.312C>A p.(Tyr104*)	Nonsense	RP
34	F	28	Ovarian Ca	+	*RAD50*	c.2083C>T p.(Gln695*)	Nonsense	RP
35	M	46	Prostat Ca	+	*CHEK2*	c.678G>C p.Leu226Phe	Missense	RP
36	F	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.

ATM was the most affected gene, which accounted for 3.6% of the study population and 21% of pathogenic variation positive patients. This ratio was high compared to a recent study that reported pathogenic ATM variation frequency as 1.4% in 768 HCS cases. In the same study, pathogenic CHEK2 variation frequency was 3.5%, similar

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 • Bilateral breast Ca	19/118 (16%) • 2/8 (25%)	*ATM, CHEK2, MUTYH, BRIP1, BARD1, TP53, RAD51C* • *CHEK2, TP53*
	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[14]. Yadav et al.[12] 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[15,16].

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 monoallalic 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[11,17]. 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[4]. 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[18].

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[19]. In our study group, 8 of 118 (6%) breast cancer patients had synchronus or metachronus 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[20] 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[21]. 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[22].

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 inreased risk for endometrial cancer[24]. 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 life[25]. 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, exprerienced 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%, respectively[26]. 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.

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