Oncologie
DOI: 10.32604/oncologie.2020.014120
Article

The Investigation of Genotype-Phenotype Relationship in Multiple Primary Malignant Neoplasia Patients

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Abstract: Multiple primary malignant neoplasms (MPMN) are rare tumors that have attracted attention with increasing incidence rates in recent years and where cancer susceptibility genes may play a role in their etiology. In this study, it was aimed to determine the genotype-phenotype correlation in patients with MPMN. From January 2018 to January 2020, thirty patients were analyzed for 59 cancer susceptibility genes and diagnosed with MPMN, using a large multigene panel with Next Generation Sequencing technique (NGS) in Turkey. The mean age of first and second cancer diagnosis of cases were calculated as 42.5 and 49.9 (respectively). These primary cancers were frequently detected in the colon and breast, and the interval between diagnosis was 89 months. In 9 of the patients (30%); *BRCA2*, *MSH6*, *MLH1*, *MUTYH*, and *ATM* were detected as causal genes. Relatives with cancer of MPMN patients with causative gene carriers were detected in higher numbers than non-carrier. According to the logistic regression model applied, patients with at least 1 relatives with cancer were found to have a 0.38-fold increased risk of being a causal gene variant carrier. Hereditary cancer susceptibility genes may play an important role in the etiology of MPMN. In MPMN cases, detection of the causal gene by genetic analysis; It will enable not only to ensure a complete and accurate diagnosis of the sick individual and to plan the treatment properly, but also to include the carriers' relatives in the intensive cancer screening, monitoring, and prevention program.

Keywords: Multiple primary malignant neoplasms; hereditary cancer susceptibility genes; next generation sequencing; family history

1 Introduction

Multiple primary malignant neoplasias (MPMN) is the occurrence of two or more primary neoplasias originating from different anatomic regions and/or different histological or morphological tissues of the same anatomical region at the same time or within a certain period of time [1,2]. The incidence of MPMN has been reported as about 0.7% to 17% [1–5]. MPMN, according to the time interval of appearance of other primer tumors; those diagnosed in the first six months are called synchronous, and those after six months are called metachronous [6]. Although some studies in the literature have shown longer life expectancy and slower cancer prognosis in MPMN compared to single primary neoplasms, the some studies MPMNs have shown worse behavior and poor prognosis [1,6–8]. Factors involved in the emergence of MPMN can be listed as genetic, immunological, environmental (smoking, alcohol consumption, endogenous or exogenous estrogen exposure, diet, obesity, physical inactivity, occupational exposure), and iatrogenic (chemotherapy, ionizing radiation) [1]. Among the genetic causes, familial cancer syndromes and genetic susceptibility conditions are the most emphasized factors [3,9]. However, the genetic etiology of MPMN has many unexplained aspects. In less than 25% of

cases, a germline causal gene has been reported [7]. Somatic mutations are known to play a role in MPMN etiology, as in single primary cancers [10]. It is important to clarify that genetic cause due to the benefits it provides in many respects such as providing appropriate genetic counseling for the patient and the family, obtaining possible clues in terms of prognosis, evaluating individualized treatment methods, and applying it. In this study, patients with MPMN who were evaluated for the elucidation of genetic etiology and diagnosed with possible new germline variants with the new generation sequencing technique were presented with their general demographic and clinical features.

The aim is to illuminate the phenotype-genotype correlation in patients with MPMN, to evaluate the cases in terms of possible treatment options, to provide a holistic approach to families in terms of genetic counseling and screening indications.

2 Materials and Methods

2.1 Data of Patients

Patients with MPMN who were evaluated for hereditary cancer syndrome in Ankara Diskapi Yildirim Beyazit Training and Research Hospital Medical Genetics Department between January 2018 and January 2020 were included in this retrospective cohort study. Adult patients with histologically MPMN diagnosed, synchronous/metachronous primary tumors were included in the study. Individuals under 18 years of age and also cases with relapsed neoplasias or metastatic cancers were excluded. As a result of the exclusion and inclusion criteria, general demographic features, family history-pedigree analysis, clinical and pathological findings, and genetic analyzes were obtained from the patient files retrospectively.

2.1 Genetic Testing

QIAcube® automated isolation system (Qiagen Inc., Mississauga, Canada) was used to extract DNA from peripheral venous blood samples. The samples whose quality and concentration (OD260/OD280, 1.8 to 2.0) were evaluated by spectrophotometric were included in the next-generation sequencing study. This study was performed on the Illumina MiSeq system platform (Illumina Inc., San Diego, CA, USA) and using the Qiagen large hereditary cancer panel (Qiagen, Hilden, Germany) kit. The genes investigated are: "*AIP, APC, ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, EPCAM, FAM175A, FANCC, FLCN, GALNT12, GEN1, GPC3, GREM1, HOXB13, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PALLD, PIK3CA, PMS1, PMS2, POLD1, PRSS1, PTCH1, PTEN, RAD50, RAD51B, RAD51C, RAD51D, RET, RINT1, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, VHL, XRCC2".* QIAGEN Clinical Insight (QCI™) Analyze software (QIAGEN, Hilden, Germany) software was used to analyze the data obtained by the NGS method. In the study, the determined variants were classified based on the criteria in the ACMG guideline [11]. Package for Social Sciences (SPSS v 15.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis and the statistical significance level was accepted as $p < 0.05$.

3 Results

The number of patients included in the study is 30, 28 were female (93.3%) and 2 were male (6.7%). The average age of first cancer diagnosis was calculated as 42.5 (range 28–64) and the average age of second cancer diagnosis was 49.9 (range 29–75). Colon cancer (9 patients, 30%) and breast cancer (8 patients, 26%) were the most common cancers diagnosed in patients, and the mean ages of diagnosis of these cancers were 43.8 and 38.8, respectively. Breast (10 patients, 33.3%) and colon (5 patients, 16.6%) cancers were the most common in the second diagnosed primary cancers, and the mean ages of diagnosis were 52.8 and 57.6. In the study, there were two patients with 3 primary cancers and the mean age of diagnosis of these third cancers was 56 (53 and 59). For all patients; the interval between the first and second cancer diagnoses was calculated as 89 months (range 4–396).

MPMN patients according to the time of diagnosis of the second primary cancer; it was classified as synchronous (≤6 months) and metachronous (>6 months) and synchronous MPMN (3.3%) was detected

in only one patient. As a result of gene analysis, pathogenic and likely pathogenic gene variants were detected in 9 patients (30%) and their genetic background was illuminated (Tab. 1).

Table 1: Hereditary cancer genes analysis results and details from the study group

Patient ID	Age	Sex	DXs	Ages at DXs	Family History of Cancer	Gene	Nucleotide/Protein Change	Loc.	ACMG S.	Zyg.	Func	dbSNP
P1	66	F	Endometrium/ Colon	44/61	2Endometrium, 1Colon, 1Cervix, 1Pharynx, 1Brain	MSH6	NM_000179.2(MSH6): c.3836_3837delGCinsA (p.Ser1279AsnfsTer48)	Ex9	PAT	Het	FS	Novel
P2	71	F	Colon/Breast	64/66	-	-	-	-	-		-	-
P3	53	F	Colon/ Endometrium	47/50	1 Colon,1 Breast,1Leukemia	BRIP1	NM_032043(BRIP1):c.2830C>G (p.Gln944Glu)	Ex10	VUS	Het	M	rs140233356
						PALLD	NM_016081.4(PALLD): c.671_672delTGinsCA (p.Met224Thr)	Ex2	VUS		M	Novel
						PMS1	NM_000534.4(PMS1) :c.1856+5G>T (p.?)	Int9	VUS		SE	Novel
						ATR	NM_001184.4(ATR): c.1732+4A>G	Int7	VUS		SE	Novel
P4	43	F	Cervix/ Parathy	33/38	1Colon, 1Thyroid, 1Prostate	-	-	-	-	-	-	-
P5	57	M	Prostate/ Colon	55/56	1Colon, 1Pancreas, 1Prostate	MSH2	NM_000251.2(MSH2) :c.-107C>A	Ex1 5'UTR	VUS	Het	RE	Novel
P6	37	F	Colon/Breast	35/34	1 Lenfoma, 1Colon	MUTYH	NM_001128425.1(MUTYH): c.217G>A (p.Glu73Lys)	Ex 3	VUS	Het	M	Novel
							NM_005732.4(RAD50):c.2840T>C (p.Ile947Thr)	Ex18				rs150401251
P7	62	F	Colon/Breast	48/49	3Breast, 1Endometrium, 1Breast+Hodgkin, 1Liver,1Stomach, 1Skin,1Larynx, 1Leukemia	AXIN2	NM_004655.4(AXIN2): c.1975C>T (p.Arg659Trp)	Ex8	VUS	Het	M	rs142670753
						BRIP1	NM_032043.2(BRIP1): c.326A>G (p.Asn109Ser)	Ex4				Novel
P8	56	F	Endometrium/ Colon /Breast	49/51/ 53	2Stomach,1 Breast, 1Colon, 1Lung	BRCA2	NM_000059.3(BRCA2):c.810_811 delAGinsCTGTTAAATG AATTT (p.Gly271CysfsTer9)	Ex10	PAT	Het	FS	Novel
P9	31	F	Colon/Thyroid	28/29	1 Breast, 2 Colon	-	-	-	-	-	-	-
P10	67	F	Ovarian/ Colon	46/65	1 Skin	-	-	-	-	-	-	-
P11	51	M	Colon/Sp.Cord	38/49	-	-	-	-	-	-	-	-
P12	42	F	Colon/Pancreas	35/40	4 Colon, 2 Endometrium, 1 Stomach	MLH1	NM_000249(MLH1).3:c.793C>T (p.Arg265Cys)	Ex10	PAT	Het	M	rs63751194
						MSH6	NM_000179.2(MSH6):c.926C>G (p.Ser309Cys)	Ex4	VUS		M	rs544222338
						PALB2	NM_024675.3(PALB2):c.3306C>G (p.Ser1102Arg)	Ex12	VUS		M	rs515726112
P13	57	F	Breast/Colon	40/55	2 Lung, 1Endometrium, 1 Stomach,1Larynx, 3Breast	BRCA2	NM_000059.3(BRCA2): c.1773_1776delTTAT (p.Ile591MetfsTer22)	Ex10	PAT	Het	FS	Novel
						MSH6	NM_000179.2(MSH6):c.3820G>T (p.Glu1274Ter)	Ex 9	L. PAT		N	Novel
P14	67	F	Colon/Breast	55/65	3 Colon, 2 Lung, 1Endometrium+Colon+Breast	MLH1	NM_000249.3(MLH1):c.676C>T (p.Arg226Ter)	Ex 8	PAT	Het	N	rs63751615
P15	55	F	Breast/Thyroid	45/50	1 Prostate,1Colon	BRCA2	NM_000059.3(BRCA2):c.977G>A (p.Ser326Asn)	Ex10	VUS	Het	M	Novel
						MSH2	NM_000251.2(MSH2):c.775C>T (p.Pro259Ser)	Ex 4	VUS			rs587781294
P16	54	F	Skin/Lym	45/48	1 Leukemia, 3Lung, 1Breast, 1Osteosarcoma, 1 Skin, 1Endometrium, 1 Colon	PMS2	NM_000535.7(PMS2):c.1490G>A (p.Gly497Asp)	Ex11	VUS	Het	M	rs199739859
						MSH6	NM_000179.2(MSH6):c.1463C>G (p.Thr488Ser)	Ex 4	VUS			Novel
P17	53	F	Breast/Pancreas	34/36	1Ewing Sarcoma, 1Tyroid, 5Breast, 3Colon, 2Prostate, 2Endometrium, 3Lung, 4Stomach	BRCA2	NM_000059.3(BRCA2):c.536_537insT (p.Ile180TyrfsTer3)	Ex 7	PAT	Het	FS	Novel
						ATM	NM_000051.3(ATM):c.5065C>T (p.Gln1689Ter)	Ex34	PAT	Het	N	rs1555104609

P18	63	F	Colon/Breast	45/56	3Lung,1 Liver,3 Bile ducts, 1Breast,1Liver	MUTYH	NM_001128425.1(MUTYH):c.884C>T (p.Pro295Leu)	Ex10	L. PAT	Hom	M	rs374950566
P19	40	F	Breast/Thyroid	36/38	1Breast	CHEK2	NM_001005735.2(CHEK2): c.721+3A>T	Int 5	VUS	Het	SE	rs587782849
P20	59	F	Thyroid/ Breast/ Stomach	45/52/59	1Stomach, 1Larynx,3Lung, 2Breast	CHEK2	NM_001005735.2(CHEK2):c.499T>A (p.Cys167Ser)	Ex 4	VUS	Het	M	Novel
P21	50	F	Thyroid/ Skin	36/47	1Breast, 1Lymphoma, 1Stomach, 1Tyroid+Colon	CHEK2	NM_001005735.2(CHEK2):c.678G>C (p.Leu226Phe)	Ex 5	VUS	Het	M	Novel
P22	38	F	Thyroid/ Breast	34/36	1 Lung	-	-	-	-	-	-	-
P23	79	F	Kidney/ Lung	58/75	2Kidney,1Colon, 1Lung,1Brain	BRCA2	NM_000059.3(BRCA2): c.3397C>T (p.Pro1133Ser)	Ex11	VUS	Het	M	Novel
P24	41	F	Thyroid/ Breast	37/40	1Colon, 1Lung, 1Breast	-	-	-	-	-	-	-
P25	45	F	Breast/ Glioblastoma	29/35	1Bladder,2Stomach, 1Oral Cavity, 1Tyroid,1Lung	CDK4	NM_000075.4(CDK4):c.363G>A (p.Met121Ile)	Ex 4	VUS	Het	M	Novel
P26	65	F	Ovarian/ Breast	57/64	1Breast, 1Endometrium, 1Lung	BRCA2	NM_000059.3(BRCA2):c.7217_7218delTT (p.Phe2406CysfsTer5)	Ex 14	PAT	Het	FS	rs876659345
P27	64	F	Breast/ Ovarian	47/59	2Colon, 1Brain	BRCA2	NM_000059.3(BRCA2):c.5791C>T (p.Gln1931Ter)	Ex11	PAT	Het	N	rs80358807
P28	52	F	Breast/ Ovarian	51/45	1Colon, 1Leukemia	-	-	-	-	-	-	-
P29	40	F	Breast/Thyroid	35/38	2Endometrium, 1Tyroid	-	-	-	-	-	-	-
P30	66	F	Lym/Breast	32/65	1Larynx, 1Breast,1endometrium	BRCA2	NM_000059.3(BRCA2):c.3310A>C (p.Thr1104Pro)	Ex11	VUS	Het	M	rs80358577|

DXs, Diagnoses; Ages at DXs, Ages at diagnoses; FS, Frameshift; M, Missense; SE, Splice Effect; RE, Regulatory Effect; N, Nonsense; ACMG S., ACMG Scoring; PAT, Pathogenic; L.Pat, Likely Pathogenic; VUS, Variant of Uncertain Significance; F, Female; M, Male; Zyg, Zygosity; Parathy, Parathyroid; Lym, Lymphoma; Sp. Cord, Spinal Cord; Het, Heterozygous; Hom, Homozygous; Func, Function; Loc, Location; Ex, Exon; Int, Intron.

The mean age of first cancer diagnosis was 45.1, and the mean age of second cancer diagnosis was 54.1 for 9 patients with pathogenic variants. Variants of unknown clinical significance (VUS) were detected in 12 (40%) of the patients. No variant was detected in the genes examined in 9 cases (30%). When the pedigree analysis of the patients was examined, it was observed that only 2 patients were isolated cases, while the other patients had relatives with at least one cancer diagnosis. The average number of relatives with cancer calculated for the all patients group was 4.5 (range 0–21).

The patients were divided into 2 groups as those with and without the causative gene variant and were compared in terms of various variables. When the cases were compared in terms of positive family history, it was determined with this analysis that the number of cancer diagnosed relatives of the patients whose causal genes were detected was higher than the others. No significant difference was found in terms of other variables (Tab. 2).

Table 2: Comparison of variables according to causative gene variant carrying status

Variable (N = 30)	Group		Statistical analysis* Probability
	Causative gene variant carriers (n = 9)	Causative gene variant non-carriers (n = 21)	
The interval between ages of diagnosis (years)	10,0 [2,0–17,0]	3,0 [1,0–33,0]	0,152
Gender			
Female	9 (%100,0)	19 (%90,5)	0,873
Male	-	2 (%9,5)	
Body Mass Index	23,48 ± 2,43	23,22 ± 3,18	0,595
Chemotherapy in the first cancer treatment			
Yes	7 (%77,8)	12 (%57,1)	0,419
No	2 (%22,2)	9 (%42,9)	
Radiotherapy in the first cancer treatment			
Yes	3 (%33,3)	7 (%33,3)	1,000
No	6 (%66,7)	14 (%66,7)	
Smoking			
Yes	1 (%11,1)	3 (%14,3)	0,815
No	8 (%88,9)	18 (%85,7)	
Alcohol			
Yes	-	1 (%4,8)	0,700
No	9 (%100,0)	20 (%95,2)	
Exogenous estrogen			
Yes	-	2 (%9,5)	0,483
No	9 (%100,0)	19 (%90,5)	
Physical activity			
Yes	-	2 (%9,5)	0,483
No	9 (%100,0)	19 (%90,5)	
Living place			
City	7 (%77,8)	20 (%95,2)	0,207
Rural	2 (%22,2)	1 (%4,8)	
Chronic Disease			
Yes	2 (%22,2)	4 (%19,0)	0,842
No	7 (%77,8)	17 (%81,0)	
Number of individuals with cancer in relatives	6,0 [3,0-21,0]	3,0 [0,0-10,0]	**0,010**

*"Independent Sample-t" test (t-table value) for comparing the measurement values of two independent variables in data with normal distribution; "Mann-Whitney U" test (Z-table value) statistics were used for data that did not have a normal distribution. *" χ^2-cross tables" were used to examine the relationships between the two qualitative variables.

The relationship between the family history of cancer and the status of the causal variant carriers in cancer susceptibility genes was tested using the logistic regression model. As a result of the applied binary logistic regression model analysis; the family history of cancer was found to have a significant relationship with a causal gene variant carriers ($p < 0.05$). In the family history of cancer, it was predicted

that the risk of being a carrier of the causal gene variant associated with hereditary cancer syndromes would increase by 38% in each individual cancer case increase (Tab. 3).

Table 3: Logistic regression model based on hereditary cancer susceptibility genes carrier risk status

Variable	OR	S.H.	Z	Statistical analysis* Probability
Constant	−2.700643	0.982863	−2.747730	0.0060
Family History of Cancer	**0.386201**	0.176336	2.190146	**0.0285**
		$R^2 = 0.219$		

*Binary logistic regression, Backward: LR model was used.

4 Discussion

MPMNs can develop due to many intrinsic and extrinsic factors such as innate and acquired immune system defects and endocrine problems, occupational diseases, industrial pollution, restricted physical activity, long-term exposure to ultraviolet rays, smoking and alcohol use, etc. Patients' exposure to chemotherapeutics and radiation treatments due to their first primary cancers may play an oncogenic role in terms of MPMN development while improving survival [12]. Another factor that plays an important role in the development of MPMN is genetic predisposition. This study was conducted with a single center using a large panel containing 59 genes, and the genetic etiology of patients with MPMN was tried to be clarified.

The most common primary tumors in patients with MPMN; head and neck, breast, prostate, colorectal and gynecological malignancies, while the most common secondary primary cancers have been reported as head and neck, lung, colorectal and gynecological cancers [1,13]. Colon and breast tumors were the most common in this study. In our series, pathogenic variants in *MLH1*, *MSH2* and *MSH6* genes associated with Lynch Syndrome (LS) were detected in 4 cases. These patients were diagnosed with endometrium, colon, pancreatic and breast cancer.

LS (Hereditary Non-Polyposis Colon Cancer) is an autosomal dominant cancer predisposition that develops due to germline mutations of DNA mismatch repair (MMR) genes. LS is characterized by a marked increase in risk especially in colorectal cancer and endometrium cancer, and cancers seen at an early age in cases attract attention [14]. Studies have also shown that an increase in the risk of malignancy in other organs like the prostate, ovary, pancreas, skin, in these people [15].

Pathogenic variants of *MLH1* and *MLH2*, lead to an earlier age of onset and a higher risk of colorectal cancer when compared to pathogenic variants of *MSH6* and *PMS2* [16]. The age of onset of colorectal cancer has been frequently reported as 54–64 in *MSH6* and *PMS2* pathogenic variant carriers, and 47–66 in *MLH1* and *MSH2* pathogenic variant carriers [17]. Colon cancer was diagnosed in all of our LS patients in our study group, and their ages of diagnosis were found to be compatible with the literature. P1 which one of these cases was being followed up with a diagnosis of the endometrium and colon cancer. It has been reported in the literature that half of the women with LS with these two cancers were first diagnosed with endometrial cancer [18]. It was observed that approximately 26% of those who were first diagnosed with colon cancer developed endometrial cancer within 10 years [19]. In our case, the first cancer diagnosis was made in the endometrium, and colon cancer was diagnosed approximately 17 years later.

In this study, the gene responsible for the etiopathogenesis of the cancers in P12 and P14 cases was identified as *MLH1*. The product of this gene, *MLH1* (mutL homolog 1) protein, is involved in DNA reparation and its loss is one of the most common causes of microsatellite instability [20]. The germline mutations of this gene often cause colon cancer and rarely pancreatic cancer [21]. P12 was diagnosed with colon and pancreatic cancer at an early age, and P14 was diagnosed with colon and breast cancer after the age of 50. It has been reported in the literature that individuals with LS up to the age of 70 have an 8.6-

fold increased risk of pancreatic cancer [22]. The fact that P14 is diagnosed with breast cancer at an advanced age made it difficult to analyze the relationship of this malignancy, which is already quite common in the general population, with LS.

Analysis for P13 showed that this patient carries germline causal variants in both the *BRCA2* and *MSH6* genes. This patient was diagnosed with breast cancer at an early age. In *BRCA2* mutation carriers, the cumulative risk of breast cancer up to the age of 70 is 38-84% [23].

In the literature, cases carrying pathogenic germline variants in both LS-related genes and *BRCA1*/*2* genes are extremely rare, and the frequency of this condition is thought to be approximately 1:203,000 in Western societies [24]. Studies on the effect of LS on breast cancer risk are contradictory. According to some studies, while there is no statistical evidence that LS increases the risk of breast cancer, some authors have stated that the risk increases 2–18 times in LS families [14]. One of the hypotheses is that mutation accumulation occurs in genes in breast cancer etiology due to MMR errors. Some authors commented that LS variants cause a more aggressive phenotype in breast cancers due to high microsatellite instability. As a result, the authors stated that the LS-breast cancer relationship is not certain, and large cohorts are needed to understand the possible risk [14]. The case is given detailed information about both gene variants, possible effects of the variants, heredity mechanism, unknown directions are explained. The importance of segregation analysis for other family members has been explained and extensive information has been given about the proposed screening tests.

Pathogenic variants in the *BRCA2* gene were detected in 5 cases (P8, P13, P17, P26, P27) in our series. *BRCA1* and *BRCA2* genes are tumor suppressor genes known as breast cancer genes and cause hereditary breast-ovarian cancer syndrome (HBOC) [25]. These genes play an important role in maintaining genomic stability and provide a repair of DNA double chain fractures with the homologous recombination mechanism. Until the age of 70, the risk of developing breast cancer is about 57% (*BRCA1*) and 49% (*BRCA2*), respectively, of the mutation carrier women in the relevant genes, and has significantly increased risk compared to the population [26]. The lifetime risk of ovarian cancer can increase up to 39% in this syndrome. In addition to breast and ovarian cancers in *BRCA1/2* mutation carriers, there is an increased risk in some other cancers such as prostate, colon, and pancreatic malignancies [27].

A patient (P17) in the study who was diagnosed with breast and pancreas cancer had pathogenic variants in the *BRCA2* gene and the *ATM* gene. The *ATM* gene is an important gene that is a DNA damage response regulator and is responsible for the autosomal recessive inherited ataxia-telangiectasia syndrome. It is known that individuals carrying heterozygote germline variants of this gene have an increased risk for breast cancer [28]. For this reason, our patient was given consultancy about the HBOC and carriage of the *ATM* gene causal variant. In some studies in the literature investigating individuals carrying cancer susceptibility genes in the state of double heterozygous (DH), it was reported that these individuals were diagnosed with cancer at an early age and MPMN was more common [29–31]. DH genotypic structures and phenotypes of the P13 and P17 cases in this study were found to be compatible with the literature.

A homozygous pathogenic variant was detected in the *MUTYH* gene in a patient with colon and breast cancer in the series. Homozygous mutations of the *MUTYH* gene are responsible for familial adenomatous polyposis type 2 (FAP 2) syndrome, characterized by adult-onset multiple colorectal adenomas and adenomatous polyposis. Although it is known that affected individuals have a high risk for colorectal carcinomas, in these patients also have an increased risk of cancer in certain organs such as duodenal, ovarian, bladder, breast, endometrium, pancreas, skin, thyroid, etc. The risk of colorectal cancer in patients with *MUTYH* polyposis up to the age of 60 is 43% -63%, and the mean age of onset of this malignancy is 48 years. Although the breast cancer risks of these cases are uncertain, the mean age at diagnosis of the patients was reported to be 53 (range 45-76) [32–33]. Our patient (P18) was diagnosed with colon cancer at the age of 45 and breast cancer at the age of 56. It was thought that the genotype of the patient played a role in the development of colon cancer, but the etiopathogenesis of breast cancer could not be elucidated.

A genotype-phenotype correlation was identified in 9 cases in the series by identifying pathogenic and likely pathogenic gene variants why MPMN susceptibility. In the other 21 cases in the series, the genetic background could not be elucidated because no known or novel causal variants were detected in the screened genes. Various VUS variants were detected in the genes investigated in 12 of these cases. In these cases, as suggested in the literature, it was planned to classify the variants at 6-month intervals in accordance with the ACMG criteria and re-evaluate the genotype-phenotype correlations. With this study, MPMN cases were investigated in the Turkish population using a large panel comprising 59 genes and has been found a causal germline gene variant carrier frequency as 30% in these patients group.

4.1 Study Limitations

This study has some methodological limitations. Although 59 well-known genes have been studied with the current gene panel, most of the cancer-related genes, candidate genes, and possible somatic mutations have not been studied. Our case series is relatively small and no co-segregation analysis could be performed for all patient relatives. Information on cancers of patients' relatives was obtained only from pedigree analysis, and pathological reports of many of them could not be reached. Many environmental and individual factors that may predispose to cancer have not been investigated, both in patients and their relatives with cancer. For all these reasons, the findings obtained in this study need to be confirmed by comprehensive studies that will include more patients.

5 Conclusion

Besides this study is one of the few studies which research MPMN-genotype correlation in the literature, as far as we know, is the first study that has been realized on Turkish MPMN patients. Detecting causal genes in MPMN cases by investigating the presence of possible genetic predisposition factors in the etiopathogenesis of their disease; will ensure that patients and their relatives are guided into a close follow-up strategy.

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

Compliance with Ethical Standards: The present study involved human participants, and it was conducted considering ethical responsibilities according to the World Medical Association and the Declaration of Helsinki. The independent Ethics Committee of the Ankara Diskapi Yildirim Beyazit Training and Research Hospital approved this descriptive case series study (Document No. 2020-86/09).

Data Availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions: ND: Principal investigator, study conception and design; ND, ED: 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.

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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