Mutation Spectrum of Familial Adenomatous Polyposis Patients in Turkish Population: Identification of 3 Novel APC Mutations

Esra Arslan Ateş[1], Ceren Alavanda[2], Şenol Demir[2], Çağlayan Keklikkıran[3], Wafi Attaallah[4], Osman Cavit Özdoğan[3], Ahmet İlter Güney[2]

[1]Genetic Diseases Diagnostic Center, Marmara University Pendik Training and Research Hospital, İstanbul, Turkey
[2]Department of Medical Genetics, Marmara University School of Medicine, İstanbul, Turkey
[3]Department of Internal Medicine, Gastroenterology, Marmara University School of Medicine, İstanbul, Turkey
[4]Department of General Surgery, Marmara University School of Medicine, İstanbul, Turkey

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ABSTRACT

Background: *Familial adenomatous polyposis (OMIM #175100) and MUTYH-associated polyposis (OMIM #608456) are rare cancer-prone disorders characterized by hundreds of adenomatous polyps in the colon and rectum, which have a high probability of malignant transformation. Attenuated familial adenomatous polyposis is a variant of familial adenomatous polyposis, which is a term used for the condition in which patients have less than 100 colorectal polyps. Germline heterozygous Adenomatous polyposis coli (APC) and biallelic MUTYH (mutY DNA glycosylase) pathogenic variations are responsible for familial adenomatous polyposis and MUTYH-associated polyposis respectively. The aim of this study is to discuss the clinical manifestations of patients having pathogenic APC and MUTYH variations.*

Methods: *We included 27 probands who have more than 10 colonic polyps in this study. After evaluation of their clinical and family histories, the probands were screened for APC and MUTYH variations via next generation sequencing. The family members of the probands carrying pathogenic variations were screened via Sanger sequencing.*

Results: *Among 27 probands, pathogenic APC and MUTYH variations were detected in 3 and 6 probands respectively. In the APC gene, 3 novel truncating variations (p.Leu360*, p.Leu1489Phefs*23, and p.Leu912*) were detected in 3 unrelated probands. In the MUTYH gene, only 2 distinct pathogenic variations were detected (p.Pro295Leu and p.Glu480del) in the homozygous or compound heterozygous state.*

Conclusion: *In this study, molecular etiology was clarified in 9 familial polyposis patients. The p.Pro295Leu and p.Glu480del variations seem to be common in the Turkish population and may be considered as a first-step genetic test in Turkish familial polyposis patients showing autosomal recessive inheritance. However more studies are needed to reveal the exact frequency of these variations.*

Keywords: *Adenomatous polyposis coli, APC, MUTYH*

INTRODUCTION

Familial adenomatous polyposis (FAP; OMIM #175100) is a rare cancer-prone disease inherited in an autosomal dominant manner. It is characterized by more than hundreds to thousands of adenomatous polyps in the colon and rectum which have a high probability of malignant transformation. Attenuated FAP (AFAP) is a variant form of FAP which is a term used for the patients having less than 100 colorectal polyps (10-100 polyps), and less malignancy risk.[1] Germline *APC* mutations are responsible for FAP. *APC* is a tumor suppressor gene located on chromosome 5q21 and encodes a large scaffolding protein having important functions in cell cycle regulation, apoptosis, transcription, and cell migration.[2] *APC* is composed of 16 exons and the last exon encodes nearly 70% of *APC* protein. The frequency of FAP in the general population is approximately 1 : 8000, and shows almost complete penetrance which affects multiple generations in the family.[3]

MUTYH-associated polyposis (MAP; OMIM #608456) is an autosomal recessive familial polyposis form resulting from biallelic pathogenic variations in the *MUTYH* gene, located on chromosome 1p34. *MUTYH* encodes a DNA glycosylase that plays a key role in the base excision repair (BER) pathway.[4] The carrier frequency of the *MUTYH* gene is estimated at 1 : 45.[5] The phenotype of MAP patients varies between Lynch-like phenotype, and early-onset colorectal cancer (CRC) without polyposis, to

Corresponding author: ***Esra Arslan Ateş****, e-mail:* ***esraarslan.md@gmail.com***
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FAP-like phenotype presenting with more than 100 polyps and high risk of CRC.[6] The lifetime CRC risk is 80-90% in biallelic *MUTYH* mutation carriers. Moreover, patients have increased risk for other malignancies, like other gastrointestinal tract cancers, ovarian, bladder, and breast cancers.[7]

To date, more than 2000 pathogenic variations in *APC* gene and 100 variations in *MUTYH* gene respectively. In this study, we report the variation spectrum of *APC* and *MUTYH* genes in Turkish FAP and MAP patients to contribute in genotype–phenotype correlation.

MATERIALS AND METHODS

Patients

Twenty-seven patients having multiple colorectal polyposis referred to the medical genetics department between 2016 and 2020 were included in the study. The detailed family histories were obtained and pedigrees were drawn according to patients' declaration. The pathologic, radiologic, and endoscopic examination reports were reviewed retrospectively. All patients were informed face-to-face and written informed consents were obtained. The study was approved by the Institutional Review Board.

Genetic Analysis

From every patient, 2 mL of peripheral blood samples were collected into EDTA tubes and stored at –20°C. Genomic DNA was isolated from peripheral blood leucocytes using QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocols. All coding exons and exon–intron boundaries of *APC* and *MUTYH* genes were amplified using the Multiplicom FAP (Agilent, Calif, USA) kit. The prepared library was sequenced on the Illumina Miseq platform (Illumina Inc., San Diego, Calif, USA). The data were analyzed by Sophia DDM data analysis software. In order to call variants, sequencing data were aligned to the human reference genome, hg19. Sanger sequencing was performed on ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific, Mass, USA) after amplifying targeted regions using the designed primers for confirmation of detected variants and segregation analysis. The novel variants were classified according to American College of Medical Genetics and Genomics criteria.[8]

Main Points

- *This is the first cohort revealing the mutation spectrum and genotype–phenotype correlation of Turkish familial polyposis and patients.*
- *We report 3 novel pathogenic APC variations.*
- *We report 2 recurrent pathogenic variations in the MUTYH gene in 6 unrelated Turkish MAP families, that can be interpreted as common variations in Turkish population.*

RESULTS

Between 2017 and 2020, 27 unrelated probands (16 male and 11 female) were referred to our department because of multiple polyposis in the colon. The ages of the patients ranged between 8 and 74, and the median age was 49.

Patients were evaluated retrospectively and the clinical characteristics of the cohort have been summarized in Table 1. Sixteen out of 27 (59%) probands had a family history of polyposis whereas 5 of them reported CRC (colorectal cancer) in their first-degree relatives. However, nonpolyposis CRC was detected in 7 (26%) probands'

Table 1. Clinical Characteristics of the Probands

Feature	N (%)
Total Probands	27 (100%)
Sex	
Female	11 (38%)
Male	16 (62%)
Number of polyps	
10-30	10 (37%)
30-100	11 (41%)
>100	6 (22%)
Extracolonic manifestations	
PBC	1 (4%)
Hydronephrosis and splenomegaly	1 (4%)
Malignancy	
CRC	17 (63%)
Medulloblastoma	1 (4%)
Desmoid tumor	1 (4%)
Family History	
Polyposis only	11 (41%)
CRC only	7 (26%)
Polyposis + CRC	5 (18%)
No	4 (15%)
Molecular Findings	
APC mutation	3 (11%)
Biallelic *MUTYH* mutation	6 (26%)

CRC, colorectal carcinoma; PBC, primary biliary cholangitis.

first-degree relatives. Among 27 patients, 17 (65%) underwent surgery because of CRC. Two patients had extracolonic malignancies, and 1 patient had medulloblastoma and also had a brother with similar findings. The other patient had a history of desmoid tumor in abdomen.

In total, 5 distinct pathogenic variations (3 in *APC* and 2 in *MUTYH* genes) were detected in 9 (33%) probands. The first proband was a 48-year-old female with heterozygous *APC* c.1079T>A (p.Leu360*) variation in exon 10, who had 70-80 colonic polyps and was diagnosed as CRC at the age of 44. Her 23- and 27-year-old children, both having more than 30 polyps, were also carriers of the same variation. An 8-year old boy was referred to us because of a father who was clinically diagnosed with FAP and had died at the age of 34. His colonoscopy revealed more than 30 polyps in the colon. We detected a c.4467_4471delACATT (p.Leu1489Phefs*23) variation in exon 16 of the *APC* gene. The third proband was a 29-year-old female, clinically diagnosed with FAP at the age of 13, she also had intraabdominal desmoid tumor history. Her father, uncle, and grandmother had died due to FAP and CRC in the fourth decade of their lives. We detected heterozygous c.2735T>A (p.(Leu912*) variation in exon 16 of the *APC* gene. All 3 variations were truncating variations and not reported previously.

Biallelic pathogenic *MUTYH* variations were detected in 6 probands. Three of them had homozygous c.884C>T(p.Pro295Leu) variation, 2 had homozygous c.1437_1439delGGA (p.Glu480del) variation and one was compound heterozygous for these 2 variations. Available family members were screened for the detected variations and characteristics of the pathogenic variation carriers are presented in the Tables 2 and 3. The pedigrees of family 7 and 9 are shown in Figure 1. The cases carrying monoallelic *MUTYH* variations with colorectal polyps were screened for all *MUTYH* gene variations, and no variation was detected. However, copy number variations were not excluded.

In 18 probands, no mutation was detected in the *APC* or *MUTYH* genes. Approximately 45% (8/18) of the probands had 10-30 polyps in colon, 2 of them diagnosed as CRC. The remaining 10 patients (65%) had more than 30 polyps in the colon and 2 of them had more than 100 polyps. The pedigree analysis of 6 of the 18 mutation-negative patients suggested an autosomal recessive inheritance. We were not able to exclude copy number variations in the *APC* and *MUTYH* genes. We detected a missense variation in the *APC* gene (c.3386T>C; p.Leu1129Ser) only in

Table 2. Characteristics of APC-Related FAP Families

		Age	Sex	Number of Polyps	Malignancy	*APC* (NM_000038) Mutation	Mutation Status
Family 1	Case 1 (Proband 1)	46	F	30-100	CRC	Heterozygous c.1079T>A (p.Leu360*)	Novel
	Case 2[a]	27	F	30-100	-		
	Case 3[a]	23	M	30-100	-		
Family 2	Case 4 (Proband 2)	8	M	30-100	-	Heterozygous c.4467_4471delACATT(p.Leu1489Phefs*23)	Novel
	Case 5[b]	34	M	>100	CRC	N/A	
Family 3	Case 6 (Proband 3)	29	F	30-100	Desmoid tumor	Heterozygous c.2735T>A (p.Leu912*)	Novel

[a]Children of proband; [b]Father of proband.

Table 3. Characteristics of MAP Families

		Age	Sex	Number of Polyps	Malignancy	Consanguineous Marriage	*MUTYH* (NM_001128425) Mutation	Zygosity
Family 4	Case 7 (Proband 4)	32	F	>100	-	-	c.884C>T (p.Pro295Leu)	Homozygous
Family 5	Case 8 (Proband 5)	41	M	>100	CRC	-	c.884C>T (p.Pro295Leu)	Homozygous
	Case 9	39	M	>100	CRC			
Family 6	Case 10 (Proband 6)	38	M	30-100	CRC	+	c.1437_1439delGGA (p.Glu480del)	Homozygous
Family 7	Case 11 (Proband 7)	47	M	30-100	CRC	-	c.884C>T /c.1437_1439delGGA (p.Pro295Leu / p. Glu480del)	Compound heterozygous
	Case 12	53	M	30-100	CRC			
	Case 13	51	M	<10	-		c.1437_1439delGGA (p.Glu480del)	Heterozygous
	Case 14	44	M	0	-		c.1437_1439delGGA (p.Glu480del)	Heterozygous
Family 8	Case 15 (Proband 8)	36	F	30-100	CRC	-	c.884C>T (p.Pro295Leu)	Homozygous
	Case 16	44	F	<10	-		c.884C>T(p.Pro295Leu)	Heterozygous
Family 9	Case 17 (Proband 9)	36	M	>100	CRC	+	c.1437_1439delGGA (p.Glu480del)	Homozygous
	Case 18	53	M	>100	CRC		c.1437_1439delGGA (p.Glu480del)	Homozygous
	Case 19	57	M	<10	-		c.1437_1439delGGA (p.Glu480del)	Heterozygous
	Case 20	32	F	<10	-		c.1437_1439delGGA (p.Glu480del)	Heterozygous

1 patient, and it was interpreted as a variant of uncertain significance.

DISCUSSION

FAP and AFAP are rare conditions, characterized by more than 100 and less than 100 polyps in the colon respectively, and its estimated prevalence is 1 : 8000.[2] The underlying molecular deficiency is germline *APC* mutation with almost complete penetrance. Molecular genetic diagnosis and follow-up are very important in these families because of nearly 100% CRC risk when left untreated.[1] The majority of *APC* mutations are truncating mutations causing lack of C-terminus of the APC protein.[2] In 3 families, we detected 3 novel truncating mutations in the *APC* gene. The proband from family 1 had a c.1079T>A variation which results in a nonsense variant (p.Leu360*), causing premature stop codon in exon 10 of *APC*. She showed clinical AFAP and developed CRC at the age of 44. Her 2 children were diagnosed at earlier ages, presented with AFAP, and had not developed CRC at the time of this study. In the second family, the disease presented in a severe form in the proband's father who had died at the age of 34, hundreds of polyps and CRC had been detected in the third decade of his life. His son (our proband) had more than 100 polyps at the age of 8. The proband's molecular genetic analysis revealed a frameshift variation in exon 16 (c.4467_4471delACATT), which predicted to cause the truncation of the APC protein at a location nearer to C-terminus (p.Leu1489Phefs*23). This was an expected result, consistent with previous studies.[9] The third proband was a 29 year-old female having more than 30 polyps in the colon and a desmoid tumor in the abdomen. We detected a novel c.2735T>A (p.Leu912*) variation in the heterozygous state in the *APC* gene. Desmoid tumors are highly invasive, non-metastasizing tumors. Desmoid tumors may manifest in 10-15% of FAP cases.[10] In our study, we detected 1 of 3 (33%) FAP cases which were proved via genetic testing. However, this ratio may not reflect the real incidence due to the low number of cases in the study.

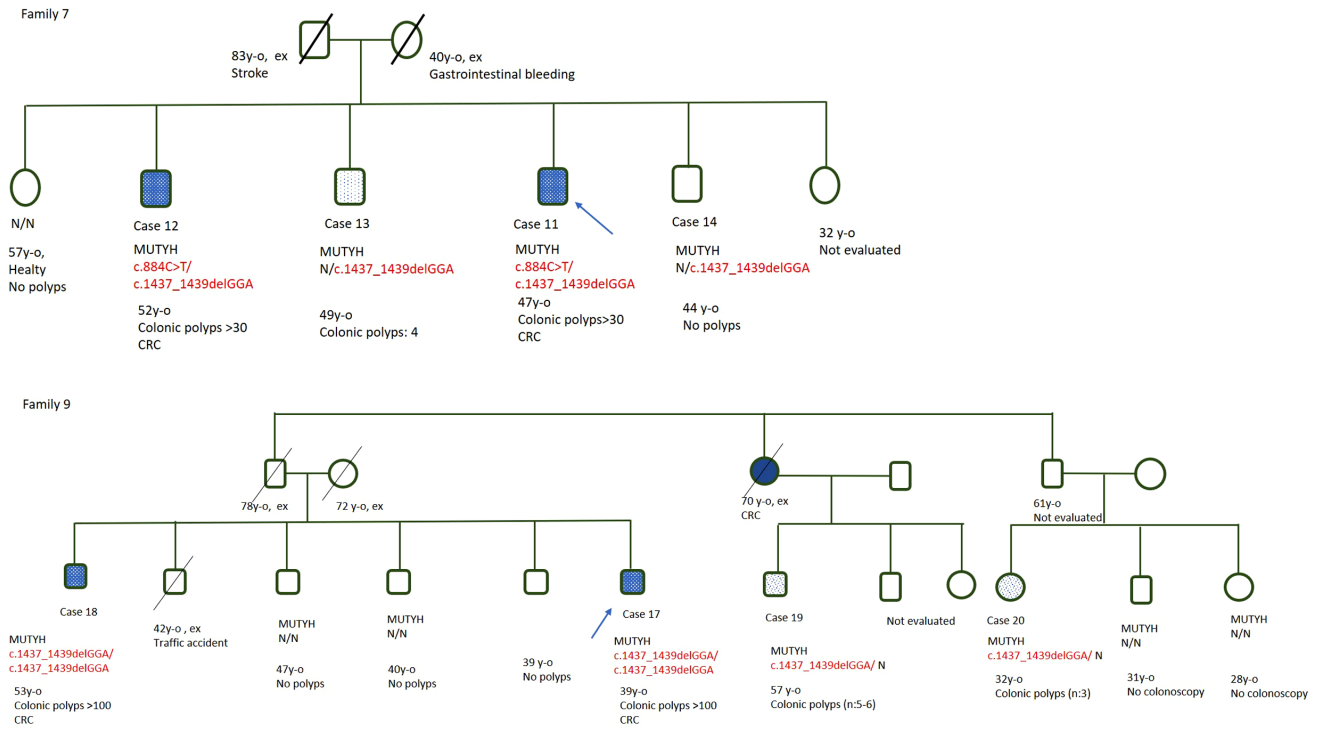

Figure 1. Pedigrees.

It was hypothesized that earlier truncations which cause null alleles may present with AFAP and the longer protein product may have a dominant negative effect. Another hypothesis to explain genotype–phenotype correlation is the presence of residual activity of *APC* causing AFAP and true null alleles causing classic FAP clinical presentation.[11] Although specific manifestations are clearly related to the mutation location, the mechanism has not been fully understood yet.

Biallelic pathogenic variations in the *MUTYH* gene were first identified in polyposis patients in 2002.[12] To date, more than a hundred distinct pathogenic variations have been reported. We identified only 2 distinct pathogenic variations in 6 unrelated families. These variations were recurrent and were reported previously in distinct studies but did not seem to be a hotspot.[13,14] We can speculate that these variations are relatively common in the Turkish population. However, all families were from the cities in the northeast of Turkey, a narrow zone (Tunceli, Elazığ, Erzurum, Rize, Bingöl). Three of the probands (50%) had more than 100 polyps in the colon and the other half had less than 100 polyps. All biallelic variation (homozygous or compound heterozygous) carriers in the same family showed the same clinical presentations regardless of the mutation types. We concluded that there is no relationship between mutation type and number of polyps but other genetic modifiers or environmental factors may have an impact on polyp number. It has been reported that half of the biallelic pathogenic *MUTYH* variation carriers had developed CRC at the time of diagnosis and the lifetime risk is unclear but seems to be quite high without treatment.[15] In our small cohort, only a 32 year-old female proband had not developed CRC at the time of diagnosis, the other 5 were older and developed CRC within the ages of 36 to 47. Molecular analysis of asymptomatic family members at risk, which we were able to screen, revealed that heterozygous *MUTYH* carriers may have polyposis with less severe presentation.

Monoallelic *MUTYH* mutations were relatively common in HNPCC-like CRC families with no mismatch repair gene defects.[16] It is known that there is a functional interaction between *MUTYH* and *MSH2/MSH6* heterodimer.[17] *MUTYH* mutation rate in the population was reported as 1.6%, and in distinct studies, the *MUTYH* mutation rate was reported as 3.7% and 4.4% in CRC cases, suggesting a causative involvement in CRC.[16,18] Similarly we detected monoallelic *MUTYH* mutation in 7 of 622 clinical exome data as a secondary finding, accounting for 1.12%.[19] However, there

are no general follow-up recommendations for monoallelic *MUTYH* carriers. We recommend colonoscopy for our patients having monoallelic *MUTYH* mutation in case of the presence of a family member with CRC. In this study, in 3 heterozygous pathogenic *MUTYH* variation carriers, we detected less than 10 polyps in the colon, and no polyps were detected in 1 case. None of the carriers had developed CRC at the time of study.

Molecular etiology could not be elucidated in approximately 70% of the polyposis families. To our knowledge, partial or total deletions of the *APC* gene are responsible for approximately 8-12% of *APC*-associated polyposis.[20,21] Intragenic *MUTYH* deletions were also reported previously.[22,23] The large deletions in *APC* and *MUTYH* genes could not be excluded and we were not able to screen the other genes responsible for familial polyposis, such as *NTHL1* and *MSH3*.

CONCLUSION

In this study, 9 polyposis families had a chance for a diagnosis and screening for possible malignancies. Because of the high malignancy risk, optimal screening programs and prophylactic colectomies are life-saving approaches for these families.

Ethics Committee Approval: The study was approved by the medical ethics committee of Marmara University School of Medicine (No: 09.2020.751).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer Review: Externally peer-reviewed.

Author Contributions: Consept – E.A.A. ; Design – E.A.A..; Supervision – O.C.Ö., A.İ.G. .; Resources – A.W. K.Ç..; Materials – A.W. K.Ç. C.A.,Ş.D.; Data Collection and/or Processing – E.A.A., C.A., Ş.D. Analysis and/or Interpretation – E.A.A., C.A., A.İ.G.; Literature Search –E.A.A., C.A., Ş.D.; Writing Manuscript – E.A.A., O.C.Ö., A.İ.G.; Critical Review – O.C.Ö., A.İ.G.

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