

New Mutations in *APC* Gene Among Familial Adenomatous Polyposis (FAP) Patients in Iran

Payam Shahnazi Gerdehsang¹, Najmeh Ranji², Mojtaba Gorji^{3*}, Safoura Pakizehkar⁴,
Ali Asghar Kiani⁵ and Saeed Veysi⁶

¹Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran
Phone: ¹<+989128135903>, E-mail: ¹<payampsq@yahoo.com>

^{2,4}Department of Biology, College of Science, Rasht Branch, Islamic Azad University, Rasht, Iran
Phone: ²<+989112407063>, ⁴<+989121944463>

E-mail: ²<najmehranji@gmail.com> ⁴<PAKIZEHKAR_S@yahoo.com>

³Department of Hematology and Oncology, Lorestan University of Medical Sciences,
School of Medicine, Khorramabad, Iran
Phone: ³<+989121948358>, E-mail: ³<mogorji@yahoo.com>

⁵Department of Immunology, Lorestan University of Medical Sciences,
School of Medicine, Khorramabad, Iran

Phone: ⁵<+989166638354>, E-mail: ⁵<aliasgharkianii@gmail.com>

⁶Young Researchers and Elite Club, Rasht Branch, Islamic Azad University, Rasht, Iran
Phone: ⁶<+989178714738> E-mail: ⁶<saeed.veysi@gmail.com>

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ABSTRACT Familial adenomatous polyposis (FAP) is a disorder by autosomal dominant inheritance caused by mutations in adenomatous polyposis coli (*APC*) gene. The aim of this paper was the investigation of a part of exon 15 of the *APC* gene in FAP patients in several provinces of Iran. Blood sample was obtained from FAP patients. A part of exon 15 of *APC* gene was amplified by PCR and underwent direct sequencing. Researchers found 23 FAP patients by severe polyposis in colorectal and identified new mutations in the three patients, including c.2910delT (17 year old) and c.3577-3578 delCA (30 and 34 year old) with severe polyposis and a substitution (N862K) in α patients. In this paper, small deletions in *APC* gene led to produce truncated non-functional *APC* protein. N862K mutation appears to be important for developing the disease. The results of this paper confirmed that there is a correlation between age of onset and phenotype with the proximity of the mutation to 5'-end of gene.

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder (Talseth-Palmer 2017). The prevalence of FAP is estimated at one per 10000 in newborns (Plawski et al. 2013). FAP is characterized by the development of hundreds to thousands of adenomas in the colon and rectum. Untreated classic form of FAP Polyps (Kerr et al. 2013) will develop to colorectal carcinoma with an average of 40 years (Wang et al. 2017). Germline mutations in the *APC* (adenomatous polyposis coli) gene are reason of FAP

disease. The *APC* gene as a tumor suppressor gene encodes a protein comprised of 2843 amino acids (Wang et al. 2017). In the *APC* protein has been determined a number of functional domains with the role of regulating several intracellular mechanisms including cell division, proliferation, adhesion, migration and cell fate determination. The *APC* domains are able to bind β -catenin, α -catenin, DLG protein, GSK-3 β , microtubule protein, Tid56, p34, Axin protein (Plawski et al. 2013). The *APC* protein is involved in free intracellular β -catenin regulation, a key component of the E-cadherin adhesion complex and the *wnt* signaling pathway (Wang et al. 2017).

The *APC* gene located in 5q21-q22 with 98-kb length (Liang et al. 2017) includes 21 exons (Masuda and Yamada 2017). Exon 15 is the largest exon comprised of more than seventy-five percent of the coding region of this gene (Vietri et al. 2010). More than 1000 mutations (Song et

*Address for correspondence:

Dr. Mojtaba Gorji

Department of Hematology and Oncology,
School of Medicine, Lorestan University of Medical
Sciences,

Khorramabad, Lorestan, Iran

E-mail: mogorji@yahoo.com

al. 2013) have been reported in *APC* gene causing FAP with a higher rate in the 5' part of exon 15 (Leoz et al. 2015). Small mutations in *APC* gene were found in about sixty to seventy percent of FAP patients, whereas large fragment deletion mutations of this gene were found in ten to fifteen percent (Sheng et al. 2010). The majority of mutations including single nucleotide alterations, small ins/del, and splice site mutations are predicted to introduce premature termination signals and subsequently produce truncated protein (Leoz et al. 2015).

The most common germline mutations in *APC* gene occurs at codon 1309 and 1061 as hotspot regions of mutations in different populations (Khan et al. 2017). In this paper, researchers investigated mutation in FAP patients with severe polyposis referred to Lorestan University of medical sciences and Firoozgar Hospital (Tehran). The aim of this paper was mutation screening in hotspot regions of *APC* gene in these FAP patients.

METHODOLOGY

Patients

Between 2015 to 2016 years, 23 related and unrelated patients with a clinical diagnosis of classic FAP have been referred to Lorestan University of Medical Sciences and Firoozgar Hospital (Tehran) for mutation screening in the *APC* gene. Diagnostic criteria were more than 100 adenomatous polyps in colon and rectum and a family history. The patients were from Lorestan, Ilam, Tehran and Mazandaran provinces. All patients gave written informed consent.

PCR-Sequencing

Peripheral blood samples were collected from FAP patients and DNA was isolated by Golden double helix kit (South Korea, Seoul). The PCR

was performed by golden double helix Kit (South Korea, Seoul) and programmed with: Initial denaturation at 95°C for 5 min, 30 cycles at 95°C for 1 min, 65°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min. The used primers were listed in Table 1. PCR products were studied by direct sequencing and analyzed by CLC main workbench v3.5 software.

RESULTS

In this paper, researchers found 23 related and unrelated FAP patients (10 women and 13 men) by severe polyposis in colorectal, while 14 patients were from Lorestan and Ilam provinces and 9 patients were from Mazandaran and Tehran provinces (Table 2, Fig. 1). A total of 4 mutations were determined by direct sequencing (Table 3) including two types of small deletions (p.Gln1193Val fsX13 and p.Ser970ArgfsX9) in 3 FAP patients (Figs. 2 and 3) with sparse polyposis and cutaneous cysts (Age of onset: At age 17-34) which has not previously been reported, a heterozygote missense mutation (p.N862K) in 1 FAP patient that asparagine was

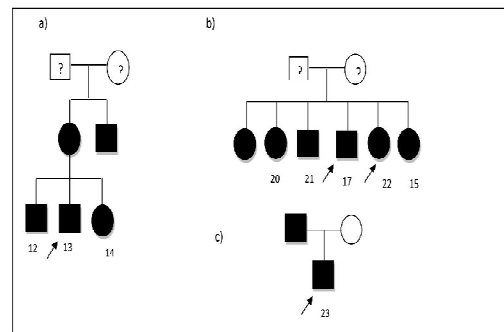


Fig. 1. Pedigrees of the 4 familial adenomatous polyposis patients with mutations (mutation detected in the patients indicated by arrows)

Table 1: Sequence of primers used for PCR

Primers name	Primers sequences	Length of production
APC-F1	5'- GAACAAAAGGAGATGTGGAATACTTGG-3'	788 bp
APC-R1	5'- TTCTGTTGCTGGATGGTAGTTGCC-3'	
APC-F2	5'-CAGTGTTACCCAGCTCCTCTTCATC -3'	794 bp
APC-R2	5'-AAAATGTGGTTGGAACCTTGAGGTGT -3'	
APC-F3	5'-CACAGAATGAAAGATGGGCAAGACC -3'	842 bp
APC-R3	5'-TGGAACCTTCGCTCACAGGATC -3'	
APC-F4	5'-GTAATCAGACGACACAGGAAGCAG -3'	579 bp
APC-R4	5'-GAACCTGGACCCTCTGAAGTCA -3'	

Table 2: Phenotype and genotype correlation in FAP patients

Patient No.	Sex	Age	Therapy	Mutation in this paper	Province
1	Female	36	Surgery	-	Lorestan
2	Male	41	Surgery	-	Lorestan
3	Female	-	Surgery	-	Lorestan
4	Female	-	Surgery	-	Lorestan
5	Female	-	Surgery	-	Lorestan
6	Male	-	Surgery	-	Lorestan
7	Male	39	Surgery	-	Lorestan
8	Male	-	Surgery	-	Lorestan
9	Male	-	Surgery	-	Lorestan
10	Male	-	-	-	Lorestan
11	Female	-	Surgery	-	Lorestan
12	Male	-	-	-	Ilam
13	Female	17	Surgery	+	Ilam
14	Male	-	Surgery	-	Tehran
15	Female	30	Laser	-	Mazandaran
16	Male	-	-	-	Ilam
17	Male	37	Laser	+	Mazandaran
18	Female	-	Surgery	-	Tehran
19	Male	-	Surgery	-	Tehran
20	Female	45	Surgery	-	Mazandaran
21	Male	43	Surgery	-	Mazandaran
22	Female	34	Surgery	+	Mazandaran
23	Male	-	Surgery	+	Tehran

Table 3: APC germline mutations in Iranian FAP patients

Patient No.	Mutation	Mutation name	Amino acid change	
9	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
11	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
13	AGTAGTAGT>AGTAGTAG	c.2910delT	p.Ser970ArgfsX9	Heterozygote
	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
14	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
17	ACAGAAACAGTC> ACAGAAA GTC (CAG>GTC)	c.3577_3578 delCA	p.Gln1193ValfsX13	Heterozygote
	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
18	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
	ACAGAAACAGTC> ACAGAAAGTC(CAG>GTC)	c.3577_3578 delCA	p.Gln1193ValfsX13	Heterozygote
22	ACT>ACG	c.4377T>G	p.T1459T	Homozygote
23	AAC>AAG	c.2586C>G	p.N862K	Heterozygote
	ACT>ACG	c.4377T>G	p.T1459T	Homozygote

replaced with lysine in codon 862 (Fig. 4), and a silent mutation in 8 FAP patients. Patient No. 13 (17 year old) with c.2910delT mutation (p.Ser970ArgfsX9) was from Ilam province and had a sick sister, brother, mother and uncle. This study's analysis had not revealed any mutations in sister and brother. Patients No.17 (37 year old) and No.22 (34 year old) were sister and brother from Mazandaran province and both had mutation c.3577_3578 delCA (p.Gln1193Val fsX13); but this mutation was not found in other sisters (Patients No.15 and No.20) and brother (Patients

No. 21). Patient No. 23 with mutation c.2586C>G (p.N862K) was from Mazandaran province and his father had died by FAP.

DISCUSSION

Investigation of *APC* gene mutations in FAP patients in different populations can be used as a tool for predictive diagnosis of subjects at risk. This tool leads to distinguish between carriers and non-carriers and administrate surveillance program for career. In this paper, 4 FAP patients showed 3 important mutations.

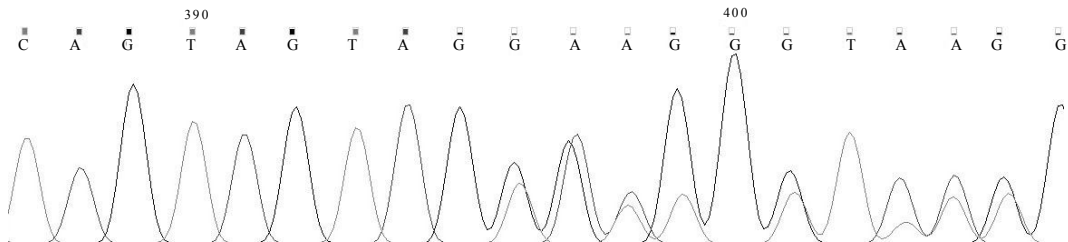


Fig. 2. Electropherograms revealed 1 bp deletion (T) in codon 970 (p.Ser970ArgfsX9, AGT>AGG) in exon 15 of APC gene. Because of frameshift after deletion, sequence was disturbed in the downstream in one FAP patient

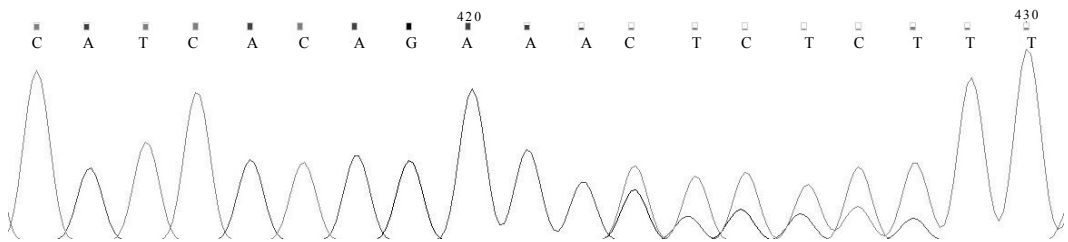


Fig. 3. Electropherograms revealed 2 bp deletion in codon 1193 (p.Gln1193Val fsX13CAG >GTC) in exon 15 of APC gene. Because of frameshift after deletion, sequence was disturbed in the downstream in 2 FAP patients

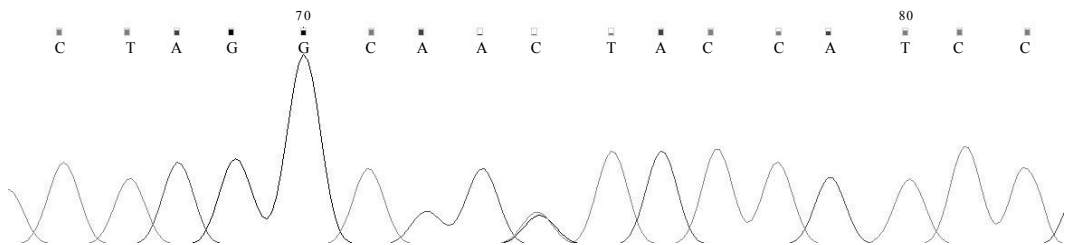


Fig. 4. Electropherograms revealed a substitution (AAC>AAG) in codon 862 (p.N862K) in exon 15 of APC gene in one FAP patient

In one patient researchers found 1bp deletion in codon 970 (p.Ser970ArgfsX9). Codon 970 locates between armadillo region and 15-nucleotide repeats in APC protein (Christie et al. 2013). β -catenin binds to three 15-nucleotide repeats (between amino acids 1020 and 1169) and 20-amino acid repeats (between amino acids 1324 and 2075) in the APC protein (Plawski et al. 2013). Loss of APC function leads to constitutive activation of β -catenin and uncontrolled cell proliferation. In this patient mentioned mutation in APC gene led to loss of important region of the gene, so that APC protein cannot be a functional protein in regulation of β -catenin and cell proliferation and as a result developing severe polyposis in the patient colorectal. Herman et al.

(2013) revealed that 3'-half of APC gene play role in chromosome segregation, apoptosis, microtubule polymerization and intercellular adhesion. Thus, in this patient loss of 3'-half of APC gene led to loss of key roles of APC protein in the cell and tumorigenesis induction.

On the other hand, herein two FAP patients had deletion in exon 15 of APC gene (c.3577_3578delCA) that led to produce a truncated protein without 20-nucleotide repeats. Only a single 20-amino acid repeat is essential for β -catenin binding to APC. Christie et al. (2013) reported that β -catenin binding to 15-amino acid sites on APC is not essential for β -catenin down-regulation. Thus, lack of 20-amino acid repeats in the APC protein may be cause of uncontrolled

proliferation and polyposis development in the two FAP patients in this paper. Also, Plawski and Slomski (2008) studied 300 unrelated Polish FAP families and found 97 mutation in 164 families such as a small deletion 4nt in codon 1193 (c.3577_3580delCAGT) in *APC* gene in FAP patients. Suraweera et al. (2006) investigated *APC* mutations in 47 patients with colorectal cancer and determined a missense mutation (S1194X) in one of the patients. These studies confirm importance of this region in development of disease. Also, in this paper was determined a missense mutation (c.2586C>G, p.N862K) in one of the FAP patients. This mutation was reported for the first time in FAP patients. Previously, Lococo et al. (2016) by next-generation sequencing on surgical specimens of 49 somatic lung cancer patients, reported p.N862K mutation in *APC* gene in a patient as a reason of carcinoma in the patient. Therefore, this mutation can influence on function of APC protein and lead to tumorigenesis progression.

Extracolonic manifestation such as desmoids, osteomas, epidermoid cysts, upper gastro-intestinal polyps and congenital hypertrophy of retinal pigment epithelium (CHRPE) have been correlated with FAP. The triad of polyposis, epidermoid cysts and osteomas is described as Gardner syndrome, a variant of FAP. Papp et al. (2016) investigated *APC* mutations in 87 unrelated FAP and AFAP (21 patients) in Budapest and reported that the risk of developing extracolonic manifestations of FAP is mostly correlated with the position of mutation in *APC* gene. In Gardner syndrome, mutations most commonly occur between codons 767–1513 of *APC* gene (Juhn and Khachemoune 2010). Gardner syndrome is associated with extra intestinal lesions such as fibromas, lipomas, sebaceous and epidermoid cysts (Talseth-Palmer 2017). Santoro et al. (2017) in an 18-month-old Gardner patient with fibroma showed that mutation Glu1464Valfs*8 in *APC* gene led to premature translation. Two FAP patients with mutation in codon 1193 (termination in codon 1206) in this paper have perfused polyposis in colorectal and epidermoid cysts. It seems that these patients have Gardner syndrome and the researchers' finding was confirmed correlation between genotype and phenotype of disease in these patients. But two FAP patients with mutation in codons 862 and 970 had polyposis without other phenotypes. It

seems that these patients were classified into classic FAP.

Aihara et al. (2014) reported correlation between genotype and the age of onset of FAP: mutations at codon 1309: at the age of 20, between codon 168 and 1580: at the age of 30, and 5' of codon 168 and 3' of codon 1580: at the age of 52. In this paper a patient with small deletion in codon 970 suffers from severe polyposis from the age of 17. Thus, it appears that the age of onset in this disease is correlated with type of mutation. So that in patient with deletion in codon 970, polyposis has been developed due to the loss of further parts of APC protein. Also, it is considerable that loss of 15-nucleotide repeats may be cause of early onset in this patient. So, in two patients with deletion in codon 1193 polyposis have been progressed after the age of 30 because of having 15-nucleotide repeats and loss of 20-nucleotide repeats. Thus, the researchers' results suggest that the presence of 15-nucleotide repeats in APC protein may decrease FAP symptoms and lead to onset of FAP after the age of 20 or 30.

On the other side, hotspot mutations 1061 and 1309 which had been previously reported in the Iranian population (Kashfi et al. 2014), were not detected in this study. Also, a silent mutation was identified in 8 patients (T1459T) that previously had been showed in other populations. In another FAP patient this mutation was not identified in the 5' half of exon 15 of *APC*, it is possible that this mutation may occur in other region of the gene or other hereditary syndromes such as MUTYH-associated polyposis (Jasperson et al. 2010).

CONCLUSION

In this paper, three FAP patients among 23 patients had new mutations (small deletions including p.Ser970ArgfsX9 and p.Gln1193ValfsX13) in *APC* gene and they produced a truncated APC protein. Also, a new mutation (p.N862K) was reported here in *APC* gene for the first time in FAP patients. It seems that the small deletions induced severe polyposis in three FAP patients because of the lack of 15- or 20-amino acid repeats in the APC protein and disruption of APC protein function. So, in one patient with N862K mutation it appears that codon 862 plays an important role in tumorigenesis, as previously reported in one lung cancer patient.

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