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Clinical and Genetic Characterization of Familial Adenomatous Polyposis: An Iranian Population Study

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Abstract

Background: Familial adenomatous polyposis (FAP) is an inherited autosomal dominant disorder, which can develop into cancer in early adulthood (100%) and is a result of germline mutations in the adenomatous polyposis coli (APC) genes.
Objectives: Identify APC germline mutations and assessed relationships between genotypes and phenotypes.
Methods: In a census-based cross-sectional study, FAP patients were selected from the referral medical centers of East Azarbaijan province between 2013 and 2016. Written informed consent was obtained from all patients for blood sampling and genetic testing. Patients undergo genetic counseling, and the pedigree was drawn. After peripheral blood sampling and DNA extraction, the potential mutation of the APC gene was detected by polymerase chain reaction (PCR), and DNA sequencing. Statistical tests were carried out using SPSS version 16.0. Categorical data between two groups were compared using the chi-square test. Independent sample t- test was used for comparison of continuous variables between two groups. The P-value < 0.05 was considered statistically significant.
Results: We identified APC gene mutations in 18 of the 30 unrelated patients with FAP (60%), including one novel frame shiftmutation, three nonsense mutations, and 14 novel missense mutations (78%). The most frequent mutations were in codon 1308 and 1350. Meanwhile, we found a novel polymorphism.
Conclusions: Our study results indicated that the APC gene has a high mutation detection rate (60%) between codons 999 and 1410, and codons 1308, and 1350 are two mutation hotspot regions.

Keywords: APC Gene, Colorectal Cancer, Correlation, Familial Adenomatous Polyposis, Genotype, Iranian Population, Phenotype

1. Background

Familial adenomatous polyposis (FAP) is an inherited autosomal disease, which can develop into cancer in early adulthood (100%) and is a result of germline mutations in the adenomatous polyposis coli (APC) genes (1). FAP has two phenotypes: classic form with more than 100 colorectal adenoma polyps and attenuated form (AFAP) with manifestations of 10-100 colorectal adenomas polyps (2, 3). Different extracolonic manifestations, including brain tumors, congenital hypertrophy of retinal pigment epithelium (CHRPE), upper gastrointestinal polyps, desmoid tumors, skin lesions, and other neoplasms may develop in patients with FAP (4-8). APC is located on the locus 5q21-22, consisting of 15 coding exons, which translate to a protein comprising 2843 amino acids (9). In the APC gene, hotspot mutation regions are located at the 5' end of exon 15 at codons 1309 and 1061, which together make up approximately 33% of all APC mutations (10). The APC gene mutational cluster region (MCR) in Chinese and Western populations have been described between codons 849 and 1376 and between codons 1250 and 1464, respectively (11). The large size of the APC gene and high level of allelic heterogeneity are challenges of clinical laboratory tests for germline mutations in APC (3). The human gene mutation database (HGMD; accessed on February 2017) has introduced more than 1800 unique APC germline mutations. However, novel mutations are regularly found in continuing studies, caused by both proband ethnicity and nature of the gene itself (12, 13).

2. Objectives

In this study, we investigated 30 unrelated patients with FAP to identify APC germline mutations and assess the possible relationships between genotypes and phenotypes for the first time in East Azerbaijan Province-Iran.

3. Methods

3.1. Patients

In a census-based cross-sectional study, FAP patients were selected from the referral medical centers of East Azarbaijan province, Iran, between 2013 and 2016. Written informed consent was obtained from all patients for blood sampling and genetic testing. Patients undergo genetic counseling and pedigree were drawn. For unrelated FAP Patients, peripheral blood samples (3 mL) were taken in EDTA containing tubes and were stored at -80°C until DNA extraction. For clinical purposes, patients were classified by a gastroenterologist into two groups including attenuated FAP (presence of 10 to 100 adenoma type of polyps) and classic FAP (over 100 adenoma type of polyps). The flow chart of the patients was presented in Figure 1.

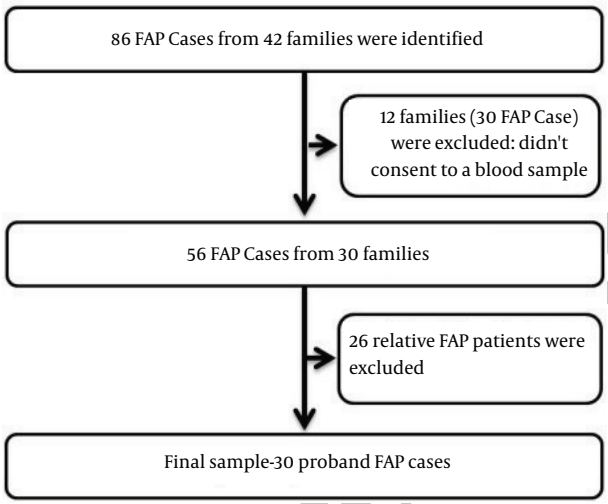

Figure 1. Flowchart of the patients studied.

3.2. Mutation Analysis

Genomic DNA was extracted from EDTA anti-coagulant peripheral blood by the YTA kit (No.YT9040-Favor Gen - Taiwan) according to the manufacturer's instructions. To detect APC mutations, the mutation region (codon 999 - 1410) was analyzed. Two pairs of primers (Gen Fanavaran Co. Iran) were designed based on the sequences published by Miyoshi et al. (1992) (14). To amplify a 1.2-kb region of exon 15; There were two overlapping PCR fragments. These primers cover more than 70% MCR in Western countries, MCR in the Chinese population, and between codons 849 and 1376 of the APC gene. Primers used are shown below: exon 15:

1) sense: 5' TCAATACCCAGCCGACCT 3', antisense- 5'CAGCTGATGACAAAGATGAT3'.

2) sense- 5'GTAAGCCAGTCTTTGTGTC3', antisense- 5'ATGGTTCACTCTGAACGGA 3'.

The reaction was carried out in 25 μL volumes containing one microliter (100 ng) of genomic DNA, 5 microliter Taq DNA Polymerase Master Mix RED (AMPLIQON- AMITIS GEN CO.) and 10 pmol of each primer. The Thermocycler (Eppendorf AG, Germany) program included a hot start denaturation step at 95°C for 5 minutes, then 35 amplification cycles of denaturation at 95°C for 45 seconds, annealing at 57°C for exon 15 (1), 59°C for exons 15 (2) for 60 seconds, and elongation at 72°C for 45 seconds, followed by a final extension at 72°C for 7 minutes. After amplification, the purified PCR product was sequenced (Faza Pajooh Co. Iran), and sequence analyses Chromatogram were performed by Finch TV 1.4 software (Geospiza, Inc., Seattle, WA, USA). We excluded sequence reads with mapping quality value (MQV) less than 30 (15, 16). For identifying de novo mutations, we check them in two databases; universal mutation database (UMD) and human gene mutation database (HGMD).

3.3. Statistical Analysis

Statistical tests were carried out using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov Smirnov test was used to determine data distribution. Categorical data between two groups were compared using the chi-square test. Independent sample t-test was used to compare continuous variables between two groups. The P-value < 0.05 was considered statistically significant.

The study was approved by the research ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1394.210), Tabriz, Iran.

4. Results

Thirty patients were clinically diagnosed as having FAP, with the mean age of 31.77 years at disease onset (range, 17 - 60 years); also, 63.3% of the subjects were male. We identified 21 (70%) patients with classic FAP, seven of whom (33.3%) had no detectable mutations in APC. Thirteen FAP patients had coexisting colorectal cancer (CRC), seven of whom had APC mutations and six had no detectable APC mutations. Based on the findings, the most common extracolonic manifestation (ECM) was congenital hypertrophy of retinal pigment epithelium (CHRPE). It was reported in nine patients (30%), five of them were APC positive and four had no detectable APC mutations. The most common symptoms included gastrointestinal bleeding (40%); an APC mutation was detected in six patients (50%).Comparison of phenotypes of FAP with and without APC mutations

revealed no significant relationship (Table 1). Genomic DNA sequencing of codon 999 to 1410 of APC gene was identified in 18 out of 30 unrelated patients with FAP, with a mutation rate of 60% (Table 2), including one novel frameshift mutation (6%; C.3416del A, P. K1139SfsX26), three nonsense mutations (17%; G4069T; P. G1357X, A3595T; p. K1199X and A4108T; p.K1370X), and 14 missense mutations (78%). Therefore, it was predicted that 22% of the mutations would cause truncation of the APC protein. Mutations at four sites were found in more than one FAP patients. The most frequent mutations were the heterozygous substitution A > G (AAA → GAA), which causes replacement of lysine with glutamine. These mutations occurred in codons 1308 (28%) and 1350 (22%). Two nonsense mutations were known, besides 16 (89%) novel mutations, as reported in the Universal Mutation Database (http://www.umd.be/APC/) and HGMD (http://www.hgmd.cf.ac.uk/ac/APC). Meanwhile, we found a novel heterozygote polymorphism in the two unrelated patients (C.3885 A>G, p.Glu1295Glu).

Table 1. Comparison of FAP Phenotypes Among Patients with and without APC Mutations

Phenotypes	No. of Patients (%)		P Value
	APC Mutation Identified	APC Mutation Not Identified	
Total no. patients	18 (60)	12 (40)	
Onset (year)			0.2[a]
Mean	31.44 (18 - 51)	32.25 (17 - 60)	
Gender			0.6[b]
Male	12 (63.2)	7 (36.8)	
Female	6 (54.5)	5 (45.5)	
FAP type			0.2[b]
Classic FAP	14 (66.7)	7 (33.3)	
Attenuated FAP	4 (44.4)	5 (55.6)	
ECM			0.8[b]
Gastroduodenal polyps	2 (66.7)	1 (33.3)	
CHRPE	5 (55.6)	4 (44.4)	
Desmoid tumor	0 (0)	1 (100)	
Lipoma	0 (0)	1 (0)	
Brain tumor	1 (100)	0 (0)	
Colorectal cancer	7 (53.8)	6 (46.2)	0.4[b]
First symptom			0.08[b]
Abdominal pain	9 (81.8)	2 (18.2)	
Gastrointestinal bleeding	6 (50)	6 (50)	
Asymptomatic	3 (42.9)	4 (57.1)	

Abbreviations: APC, adenomatous polyposis coli; CHRPE, congenital hypertrophy of retinal pigment epithelium; ECM, extracolonic manifestations; FAP, familial adenomatous polyposis.
[a] t-test.
[b] Chi-square.

5. Discussion

The genetic basis of classic and attenuated APC-associated colorectal adenomatous polyposis is a heterozygous germline mutation of the APC gene (17). In nearly 60 - 70% of FAP patients, micro mutations of APC gene were found. For determining the APC gene micro-mutations, direct DNA sequencing is the most reliable approach (18). Although studies have already been conducted on the germline APC mutation, information in the Iranian population is limited and scattered (19-21). This is a result of misdiagnosis and lack of laboratory facilities.

In the present study, the mutation rate was 60% higher than previous rates reported in Iran (27%) and Korea (33.3%), and was slightly higher than that reported in China (50%) (9, 22, 23). This difference in mutation rates might have resulted from different detection methods, sample size, and human subjects.

Studies conducted on Iranian populations from Northeast, Southwest, Central, and Northwest of Iran (the present study) indicates molecular heterogeneity in the APC gene (19-21).

5.1. Genotypes and Phenotypes

Although the association between germline APC and FAP phenotypes is well established, further definitions are necessary (24-26). We observed that a mutation in codon 1199 was associated with classic FAP, early disease onset, and prediction of truncated proteins. These features correlate with another previous report (Michils et al. in 2002) (27). The mutations in codons 1327, 1328, and 1385 were novel and related to the youngest age at diagnosis, reinforcing the notion that this region is associated with a classic phenotype. In this study, the mutations which predicted the results in the truncated protein were observed in classic FAP patients, which probably showed an association between the genotype and severity of polyposis (26). The mutations in codons 1308 (28%) and 1350 (22%) were observed in most patients in this study; therefore, they can be hotspot mutations in Iran. It is interesting that all those who had mutations in codon 1350 showed abdominal pain as the first symptom. We detected four attenuated forms of FAP with mutations in areas reportedly associated with the classic forms (between codons 1250 and 1464) (28). This finding agrees with previous studies and emphasizes on the need to include these areas in mutational screening of APC gene among AFAP patients (25). Extracolonic manifes-

Table 2. Clinical Phenotypes and Germline APC Mutations in Iranian (A) Patients with FAP

Patient ID	DNA Change	Protein Changes	Mutation Type	FAP Type	Onset (year)	First Symptom	ECM	CRC	Known/New
1	3416delA	Lys1139Serfs*26	Frameshift	C	40	AP	CHRPE	+	New
7	3595 A>G	Lys1199Glut/acid	Missense	C	31	AP	GDP	+	New
16	3595 A>T	Lys1199 stop	Nonsense	C	25	AS	CHRPE	-	New
8	3868 A>G	Asn1290Asp	Missense	C	28	AS	-	-	New
1, 8, 9, 17, 28	3922 A > G	Lys1308Glut/acid	Missense	C,C,A,C,A	40,28,40,28,23	AP,AS,GB,AP,GB	CHRPE ,-,-,-,-	+,-, +, +, -	New
5	3981 A > T	Ser1327Ser	Silent	C	19	GB	-	-	New
5, 1	3982 C > G	Gln1328Glut/acid	Missense	C,C	19,40	GB,AP	-, CHRPE	-, +	New
2	3998 A > G	Lys1333Arg	Missense	C	42	AP	CHRPE	+	New
28	4023 T > A	Ser1341Arg	Missense	A	23	GB	-	-	New
26	4046 A>G	His1349Arg	Missense	C	29	AP	-	-	New
19, 22, 26, 23	4048 A>G	Lys1350Glut/acid	Missense	A,C,C,C	51,20,29,44	AP,AP,AP,AP	GDP,-,-, CHRPE	-, +, -, +	New
22	4069 G>T	Gly1357 stop	Nonsense	C	20	AP	-	+	Known
18	4108 A>T	Lys1370 stop	Nonsense	C	42	GB	CHRPE	-	Known
22	4137 G>A	Glx1379Glx	Silent	C	20	AP	-	+	New
6	4148 T>C	Met1383Thr	Missense	C	28	GB	-	-	New
27	4155 C>G	Ser1385Arg	Missense	C	18	AP	-	-	New
12	4164 T>A	Thr1388Thr	Silent	A	34	GB	-	-	New
25	4176 A>G	Ser1392Ser	Silent	C	24	AP	Brain tumor	-	New

Abbreviations: A, attenuated FAP; AP, abdominal pain; APC, adenomatous polyposis coli; AS, asymptomatic; C, classic FAP; CHRPE, congenital hypertrophy of retinal pigment epithelium; CRC, colorectal cancer; ECM, extracolonic manifestations; FAP, familial adenomatous polyposis; GB, gastrointestinal bleeding; GDP, gastroduodenal polyps.

tations were identified in 15 (50%) out of 30 patients. This study showed a lower frequency of ECM in patients with APC-positive mutations (8/18; 44%) than those with APC-negative mutations (7/12; 58%). The most common ECM was CHRPE (nine patients), followed by gastroduodenal polyp (three patients), desmoid tumor (one patient), and lipoma (one patient). One patient had undergone an operation due to a brain tumor (schwannoma). Five out of 9 patients who presented with CHRPE had a detectable APC mutation between codons 1139 and 1370 and showed severe polyposis (Table 2). The location of the mutation in CHRPE was consistent with a previous report (29). Overall, there are controversies regarding the relationship between the severity of upper gastrointestinal polyposis and germline APC genotype (30, 31). In patients with classic and attenuated FAP with gastroduodenal polyps, APC gene mutations were in codons 1199 and 1350, respectively. These regions were inside the area reported previously (32). The relationship of colorectal polyposis with the brain tumors is attributed to Turcot's syndrome. We observed that a young girl with classic FAP had a mutation in codon 1392. She had an unusual brain tumor located in the cerebellopontine angle. The pathological diagnosis was the schwannoma. However, the relationship between this mutation and schwannoma should be further investigated. The mutation in the desmoid tumor was beyond codon 1403 (25, 29); therefore, we did not find any mutation in the desmoid tumor. The mutations in codons 1139, 1199, 1328, 1333, 1350, and 1357 were responsible for the development of CRC (6/18) in the classic FAP phenotype, while the mutation in codon 1308 was responsible for the development of CRC in both classic and attenuated FAP. All of these mutations are novel, except for the mutation in codon 1357, which was previously described by Van der Luijt and colleagues, although they did not discuss colon cancer (33). This study had some limitations; the inability to find any "large rearrangements" in APC gene because these deletions or duplications are not identifiable by conventional sequencing techniques (34). We could not calculate associations between clinical variables and the identified APC germline mutations, a limitation of this study as the small sample size.

5.2. Conclusion

In conclusion, our study results indicate that the APC gene has a high mutation detection rate (60%) between

codons 999 and 1410, and codons 1308 and 1350 are two mutation hotspot regions in Iranian patients with FAP.

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