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Identification of large deletions in the *APC* gene in Russian patients with familial adenomatous polyposis

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Familial adenomatous polyposis (FAP) is a hereditary syndrome characterized by the presence of multiple adenomatous polyps in the colon. The main cause of the disease is a germline mutation in the *APC* gene. Here we report 4 unrelated FAP patients with different large deletions in the *APC* gene detected by Multiplex Ligation-dependent Probe Amplification (MLPA) method: deletion of exons 7-15, deletion of promoters B, A, and 5'-UTR region and deletion of promoter B (in 2 patients). The deletion of promoters B, A, and 5'-UTR was not described in the literature earlier, so we report it for the first time. In 2 families with promoter B deletion, we could identify the tendency for decreasing the age of disease manifestation in each next generation, in contrast to the previous one. The incidence of large deletions in *APC* among Russian patients with FAP reached 4.8% and our finding suggests the need to study this gene by MLPA in "no mutation patients" after Sanger's sequencing.

Key words: familial adenomatous polyposis, large deletions, hereditary colorectal cancer, APC gene, germline mutations

Familial adenomatous polyposis (FAP) is a syndrome with autosomal dominant inheritance, causing the presence of tens, hundreds, and sometimes thousands of adenomatous polyps in the colon with 100% risk of transformation into colorectal cancer (CRC) without adequate surgical treatment [1]. Up to 1% of all colorectal cancer cases are caused by this syndrome [2]. The incidence of FAP in Europeans is up to 1:10000 [1]. The classic form of the disease is characterized by the presence of more than 100 adenomatous polyps in the colon and the age of cancer development less than 45 years old. On the contrary, the attenuated form of the disease is associated with a lower number of polyps (<100), and later age of colorectal cancer manifestation (>50 years old) [3, 4]. The most common form of FAP is the classic form, which exists in more than 90% of patients [5]. Along with colorectal cancer in patients with FAP, other organs can be affected: stomach, duodenum, thyroid gland, brain, and liver, besides desmoid tumors, can occur. All this necessitates lifelong clinical monitoring of target organs for timely diagnosis of possible cancer at the early stages [6].

The main cause of the disease is a germline mutation in the *APC* gene (Adenomatous Polyposis Coli), which can be detected in 65–80% of patients with FAP [5, 7]. This gene is located on chromosome 5 in the region 5q21-q22 and consists of 138,742 bp. The main transcript of the *APC* gene (NM_000038) includes 16 exons, fifteen of which encode a protein consisting of 2843 amino acids. APC protein plays an essential role in adhesion, cell migration, apoptosis, etc., but its main function as a tumor suppressor is β-catenin protein binding and regulation of the WNT pathway [8, 9]. Currently, more than 1,650 unique germline variants have been identified in *APC* gene according to the InSiGHT DNA Variant Database; and 1975 variants – according to the HGMD® Professional 2019.2 database.

The most common pathogenic mutations in the *APC* gene are deletions c.3927_3931del and c.3183_3187del, which are detected in about 30% of patients with FAP. The main types of germline mutations are small deletions/insertions, as well as nonsense mutations, which are found in about 85% of cases [10]. According to HGMD® Professional 2019.2 database, mutations of splicing site (6%, 118/1975) and large deletions, exciting one or more exons (7%, 140/1975) are significantly less common. In this case, not every *APC* gene study on FAP patients involves the search for large deletions using hybridization methods in situ, Southern Blot or Multiplex Ligation-dependent Probe Amplification (MLPA). The present study is devoted to the search of large deletions in the *APC* gene by MLPA on 'non-mutated' Russian patients with classic FAP

after routine methods of conformation-sensitive electrophoresis and Sanger sequencing.

Patients and methods

The genetic investigation was conducted on 23 patients (13 females and 10 males aged from 15 to 45 years old), who were clinically diagnosed with the classic form of familial adenomatous polyposis (colonoscopy revealed more than 100 polyps). After the detection of large deletions in the *APC* gene on 4 patients, DNA diagnosis was carried out on 5 of their blood relatives (4 females and 1 male aged from 13 to 53 years old). All patients and their blood relatives gave informed consent for the clinical and genetic studies.

All 23 patients during the period from June 2013 to February 2017 were searched for germline mutations in the *APC* gene (RefSeq NM_000038.5) as the first stage by means of conformation-sensitive electrophoresis and further sequencing by the Sanger method. Since they did not have mutations, the 2nd stage of the molecular genetic investigation was carried out (March 2018 – February 2019) – the search for large deletions using the MLPA method. The DNA was isolated from peripheral blood lymphocytes by phenol-chloroform extraction [11].

Detection of large deletions/duplications in the *APC* gene was performed by Multiplex Ligation-dependent Probe Amplification (MLPA) method using a set of reagents SALSA MLPA P043-APC, Lot #D1-0513, version D1 (MRC-Holland, Netherlands) according to the manufacturer's protocol. The separation of product fragments was carried out using the genetic analyzer ABI PRISM 3130XL (Applied Biosystems, USA). The software Coffalyser.Net provided by MRC-Holland (Netherlands) was used for the data analysis.

According to the manufacturer's protocol, the presence of the detected mutation is likely to be verified by another method (without exact specifying a different method). In this case, we were guided by the criteria of the international HGMD® Professional 2019.2 database and used Real-Time PCR with EvaGreen as a confirmation method for detected mutations verifying. Several segments of the *APC* gene were amplified using the StepOnePlus (Thermo Fisher, USA) device and then we compared the number of gene copies with the segment containing large deletion to the same numbers without a mutation. The results obtained were normalized to the same number of *APC* gene copies in healthy control. As a healthy control, we used DNA samples from 62-y.o. male without colorectal pathology (according to total colonoscopy) and no oncological family history. The primers for *APC* gene amplification were: *APC*_promotor_B F: 5'-AAGCCAGCAACACCTCTCAC-3', *APC*_promotor_B R 5'-AGTACCTGGGAACAGCATCG-3'; *APC*_5exon F 5'-CATGCACCATGACTGACGTA-3', *APC*_5exon R 5'-AGCTCTTCGCTGTTTTATCACTT-3'; *APC*_6exon F 5'-GAATGATTTGACATAACCCTGAGC-3', *APC*_6 exon R 5'-CCCACAAACAAGAAAGGCAAT-3'; *APC*_9exon F 5'-CAGCACTCCACAACATCATTC-3', *APC*_9exon R 5'-GCTTTGAAACATGCACTACGA-3'. The comparative analysis was performed using the StepOnePlus V.2.2.3 software.

Results

During the DNA analysis of 23 samples from patients with familial adenomatous polyposis large deletions were revealed on 4 (17%) patients: deletion of exons 7–15 (GRCh38/hg38: g.(?_112801306)_(112844029_?)del), deletion of promoters B, A, and 5'-UTR region (GRCh38/hg38: g.(?_112707510)_(112738403_?)del) and deletion of promoter B (GRCh38/hg38: g.(?_112707510)_(112707864_?)del), which was found in 2 families (patients A108 and A181) (Table 1, Figure 1). Real-time PCR with EvaGreen verified all detected large deletions: the signal from promoter B (deleted in one allele in patient A108) was significantly lower than one from normal exon 5 in the same patient, while they were equal in healthy control. It was confirmed by the same PCR results for patient A250 with normal exon 6 and deleted exon 9 (7–15) (Figure 2).

The family history of the female patient A108 included information about the detection of colorectal tumors in 4 generations (Figure 3). Four of her relatives were diagnosed with rectal cancer, which was the cause of death in 3 cases. In each next generation, cancer arose at an earlier age: in the first generation – at the age of 54 years, in the second – at 50 and 48 years old, in the third – at 44 years old. In addition, 4 family members were diagnosed with FAP. Again, we noted that in one generation FAP manifested at the age of 44 and 40 years old, and in the next – at 34 and 25 years old. DNA diagnostics conducted in the family demonstrated the presence of promoter B deletion in the *APC* gene of the patient and her cousin with FAP. The patient's son had no mutation.

In the family history of the female patient A181, the disease is traced during 3 generations (Figure 4). Five relatives were

Table 1. Data of the patients with large deletions in the *APC* gene.

Patient	Gender	Age	Diagnosis	Mutation	Number of affected relatives
A108	female	40	FAP	deletion of promoter B	7
A181	female	27	FAP	deletion of promoter B	9
A250	male	38	FAP with CRC	deletions of exons 7–15	1
A332	male	40	FAP	deletion of promoters B, A, and 5'-UTR region	2

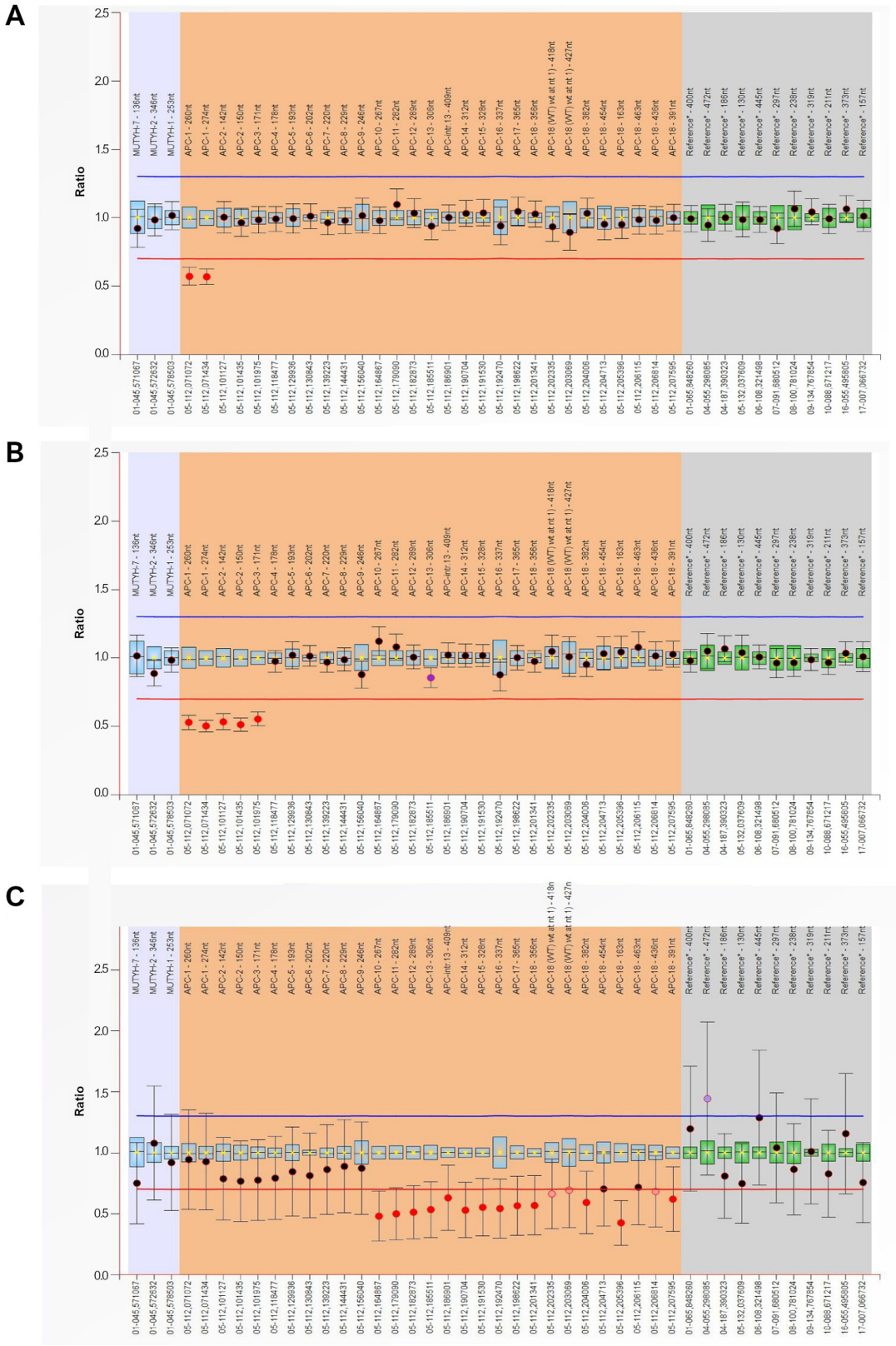

Figure 1. Detection of the deletions in the *APC* gene by the MLPA method. A) Deletion of promoter B; B) Deletion of promoters B, A, and 5'-UTR region; C) Deletion of exons 7–15.

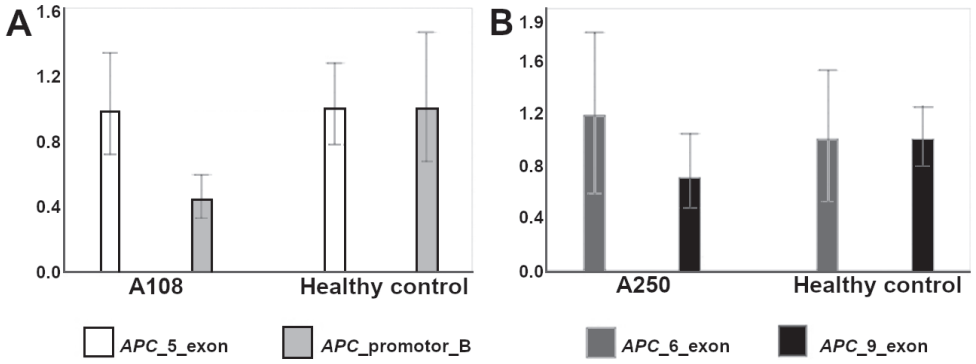

Figure 2. Comparative level of the signals from real-time PCR amplificated segments of exon 5 and promoter B (*APC* gene) in the patient A108 vs control (A) and exon 6 and exon 9 in the patient A250 vs control (B).

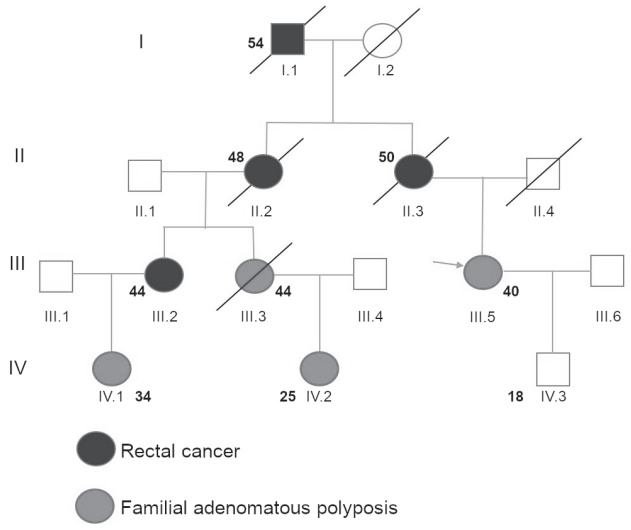

Figure 3. Pedigree of the female patient A108 (indicated by arrow) with deletion of promoter B in the *APC* gene.

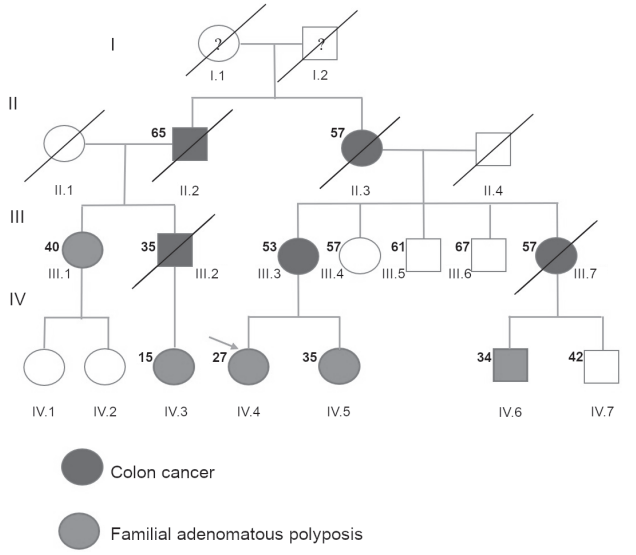

Figure 4. Pedigree of the female patient A181 (arrow) with deletion of promoter B in the *APC* gene.

diagnosed with colon cancer. At the same time, we also noted the decrease in the age of development of the disease in each next generation: of relatives from the first generation CRC appeared at the age of 65 and 57 years, and in the next generation – at 35, 53, and 57 years old. Four out of 5 patients died because of CRC. In addition, 5 relatives were diagnosed with FAP, one of whom in one generation at the age of 40 years old, and in the next generation – at 15, 27, 34, and 35 years old. DNA diagnosis demonstrated the presence of promoter B deletion in the *APC* gene in the female patient, her sister with FAP, and her mother with CRC with FAP.

A germinal mutation g.(?_112801306)_(112844029_?)del (deletion of exons 7–15) in the *APC* gene was revealed on the male patient A250 (Figure 1). The clinical diagnosis of FAP was established at the age of 32, but he refused the proposed surgical treatment at that time. At the age of 38, he was diagnosed with primary-multiple (5 tumors) synchronous cancer in the descending colon, the sigmoid colon (3 tumors), and the rectum. According to the patient`s family history, his parents had no symptoms of the disease: his father died from other causes, and his mother was alive. However, his 13-year-old daughter was found to have a similar mutation in the *APC* gene.

The male patient A332 revealed germinal mutation g.(?_112707510)_(112738403_?)del (deletion of promoters B, A, and 5'-UTR region) in the *APC* gene (Figure 1). Clinical diagnosis of FAP was established at the age of 40 when colonoscopy revealed more than 100 polyps without signs of malignancy. His family history demonstrated 2 cases of CRC: his sister was diagnosed with rectal cancer with FAP at the age of 35, and his mother died because of colon cancer at the age of 40.

Discussion

The detection rate of small germline *APC* gene mutations (without routine search for large deletions/insertions) in Russian patients with classic FAP was 72.2% (78/108) in a previous study [12]. Thus, no *APC* gene mutations were detected in 27.8% cases by traditional methods. In this study, large deletions in the *APC* gene were found almost in every sixth (17.4%; 4/23) of 'non-mutated' patients using the MLPA method. Thereby, the use of the MLPA method in Russian classic FAP patients allowed detecting additional *APC* gene mutations in 4.8% of cases. This incidence (4.8%) of large deletions in Russian patients is significantly lower than the same demonstrated in patients from Belgium (14.8%) [13], China (14.3%) [14], Sweden (12.5%) [15], Taiwan (7.6%) [16], and Spain (7.3%) [17]. At the same time, comparable frequency of large deletions detection was found in Czech (6%) [18], Iranian (5.8%) [19], and Polish (5.7%) [20] patients. On the contrary, it is necessary to point out that in some populations (Greeks and Slovaks) large deletions in the *APC* gene were not found at all [18, 21]. According to the results of another Russian study, no large deletions in the *APC* gene were also found in FAP patients, but in 1 case (1/13) an insertion in this gene (c.755_756insAGGTCATCT CAGAACAAGCATGAAACCG) was detected [22].

In our study, the germline mutations with promoter B deletion (g.(?_112707510)_(112707864_?)del) were found in 2 different families, but the boarders of those mutations were unknown, that`s why we could not consider them the same. However, it is important to note that both families had the same features: the age of manifestation of the disease was earlier in each next generation that is why we consider genetic testing for *APC* mutations to be essential in their children at the age <10 years old. All mutation carriers should undergo endoscopic examination at the age of 10–11 and, in the case of a dramatic increase in the number of adenomas or suspected malignancy of any polyps, a prophylactic surgery should be accomplished.

The mutation g.(?_112707510)_(112738403_?)del (deletion of promoters B, A, and 5'-UTR region) in a 40-year-old male included in our study did not occur in any databases and we have described it here for the first time. Unfortunately, it was impossible to confirm its pathogenic potential with the genetic diagnostic of the affected relatives, because his mother (40 years old) and his sister (36 years old) had died from FAP with CRC long before our study. At the same time, there was no decrease in the age in the onset of the disease in this family. Thus, we consider that DNA diagnostic and endoscopic examinations in this family is worth starting at the age of 18–20.

At last, the germline mutation g.(?_112801306)_(112844029_?)del (deletion of 7–15 exons) was detected in a male patient with FAP at the age of 32. His parents had no colonic polyps or neoplasm, and that might indicate a high probability of the mutation occurrence in our patient *de novo*. Unfortunately, the patient refused proposed surgery at the time of detection and 6 years later, he was diagnosed with several synchronous malignant colonic tumors.

Finally, an interesting point is that none of the 4 families with large deletions in the *APC* gene had any other pathology associated with FAP.

In conclusion, in the present study, we have demonstrated for the first time the validity of searching for large deletions in the *APC* gene by MLPA among 'non-mutated' Russian FAP patients after routine genetic methods. It additionally allowed us to detect the hereditary cause of the disease in 4.8% of patients with FAP. Besides, in families with mutation g.(?_112707510)_(112707864_?)del (deletion of promoter B) we registered an interesting feature: the age of manifestation of the disease was earlier in each next generation than previous one.

For the first time, we have discovered a new germline mutation in the *APC* gene g.(?_112707510)_(112738403_?)del, which is characterized by the deletion of promoters B, A, and 5'-UTR region, with a phenotype of classic FAP.

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