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Different phenotype manifestation of familial adenomatous polyposis in families with APC mutation at codon 1309

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Germline mutation in *APC* gene induced development of familial adenomatous polyposis (FAP). The risk of developing specific manifestation of FAP is often correlated with the position of the inherited *APC* mutation. Patients with mutations localized in the largest exon 15 between codons 1286 and 1513 (mutation cluster region, MCR) have generally a worse prognosis with early onset of the disease. We found 6 FAP families with mutation at codon 1309 (3927_3931delAAAGA) in the cohort of 39 FAP Slovak families with rapid cancer progress. In addition, mutation in codon 1309 was detected in three family members, one of them with a very different phenotype. This oldest family member, aged 81, has persisted asymptomatic without clinical manifestations.

Key words: Colorectal cancer; familial adenomatous polyposis (FAP); APC gene mutations; single strand conformation polymorphism (SSCP)

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder, caused by a germline mutation in the adenomatous polyposis coli (*APC*) gene, on chromosome 5q21. It is characterized by the development of hundreds of pre-malignant colonic polyps which, if left untreated, will eventually progress into colorectal cancer (CRC). The mean age at which adenomas develop is 15 years [1]. The incidence of FAP is one in 5,000 to 10,000 in central European population. The precancerous stage is characterized by nearly 95% penetrance and each carrier of the defected allele is affected [2].

The *APC* gene consists of 8,535 base pairs organized into 15 exons. Exon 15 contributes 70% to the open reading frame. Mutations in *APC* were first reported in 1991 [3] and to date more than 600 mutations have been identified [4]. The most common germline mutation involves the introduction of a premature stop codon, either by a frameshift mutation (68%), nonsense mutation (30%), or large deletion (2%), leading to truncation of the protein product in the C-terminal region. The majority of germline and somatic *APC* mutations occur in exon 15, and more than 50% occur between codons 1286 and 1513 – known as the mutation cluster region (MCR) [5]. Mutation hotspots are located at codons 1309 and 1061, accounting for approximately 17% and 11 % of all germline *APC* mutations, respectively [6].

In FAP patients not only colorectal adenomas but also various extracolonic manifestations are observed. These include congenital hypertrophy of the retinal pigment epithelium (CHRPE), upper gastrointestinal tumors, desmoids tumors, dental abnormalities, osteomas, lipomas, epidermoid cysts. The severity and extracolonic manifestations of FAP appear to be correlated with the site of the mutation. Mutations between codons 169 and 1393 result in classic FAP, whereas mutation in the very 5´and 3´ends of the protein as well as alternatively spliced sites in exon 9 cause an attenuated form of FAP [7, 8]. Alternations between codons 1250 and 1464 result in profuse colorectal polyposis, presenting with thousands of colorectal adenomas. Retinal lesions occur with mutations between codons 463 to 1444 [9, 10]. The *APC* mutations between codons 1403 and 1578 are associated with Gardner's syndrome [11] and between codons 1445 and 1578 with severe desmoids, osteomas, and epidermoid cysts [9].

In our research we analyzed 113 families suspected of adenomatous polyposis and we found a mutation in *APC* gene in 39 families [12]. The most common mutation at codon 1309 (3927_3931delAAAGA) was identified in six families. This mutation tends to cause a particularly severe phenotype with early onset of the disease. In this report we describe one of these six families with different clinical manifestations.

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Patients and methods

Patients. The members of one family were screened for *APC* germline mutations. Written informed consent was obtained from each patient before genetic testing.

DNA extraction and PCR amplification. Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp® DNA blood Kit (Qiagen). DNA samples were amplified using PCR mostly in the program: 5min at 94˚C, once; 1min at 94˚C; 1min at annealing temperature – 58 to 63˚C; 1min at 72˚C, 30 times; and 7 min at 72˚C, once.

Single strand conformation polymorphism (SSCP). PCRs for SSCP were performed from approximately 150-200ng of genomic DNA, 80 mM dNTP, 1mM 10x PCR buffer (Qiagen), 0.5 U of Taq polymerase (Qiagen), 10 pmol of each primer, to a total volume of PCR mixture of 25µl. The sequences of the primers used for the *APC* gene were described by Groden et al. [13]. The samples were denaturated for 5 min at 95˚C and then placed on ice for 5 min in order to prevent reannealing, loaded onto a 6% polyacrylamide gel and electrophoresed at 10˚C at 55V overnight. The gel was silver-stained as described [14].

Heteroduplex analysis (HDA). PCRs for HDA were performed as described before [14]. The samples were denaturated for 10 min at 95°C, followed by annealing for 60 min at 55°C. Heteroduplexes were then loaded on 8% non-denaturing gel, ran at 30mA/gel and silver- stained as described.

Direct DNA sequencing. Amplicons were purified by solid-phase extraction and bidirectionally sequenced with the PE Applied Biosystems Big Dye Terminator Sequencing Kit according to the manufacturer's instructions. Sequencing extensive products were analyzed on a PE Applied Biosystems ABI-PRISM 310 sequencer.

Results

The proband (Fig.1; III-1) of the family analyzed was an 18-year-old male, who underwent his first colonoscopic examination at the age of 14. This examination revealed a great number of colorectal adenomatous polyps without malignant transformation. The proband was monitored by a neurologist due to suspicion of non-progressive myopathy. Peptic ulcer occurred in the family of the proband´s father and the mother's brother (II-1) was also affected. At the age of 81, the proband´s grandfather (I-2), was a healthy old man, without any signs of FAP, including polyps. The proband´s brother was 22 years old and did not have any gastric problems. No type of cancer has been reported in the proband´s family.

We used the molecular screening methods of heteroduplex analysis (HDA), single strand conformation polymorphism and DNA sequencing to identify *APC* mutation in six members of the FAP suspected family. SSCP technique revealed extra band in segment G of exon 15 in three samples (Fig.2, lines 1, 2, 3). Abnormalities in SSCP conformation were confirmed by creation of heteroduplexes using HDA technique of the same segment (Fig.3, lines 1, 2, 3).

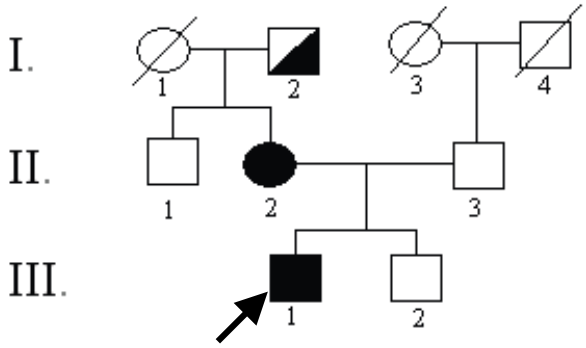

Figure 1. Pedigree of the family analyzed, suspected of adenomatous polyposis. Proband (III-1) and his mother (II-2) were diagnosed of FAP. Proband´s grandfather (I-2) is positive for *APC* mutation but he has no clinical manifestations. Proband´s brother (III-2) is negative for *APC* mutation.

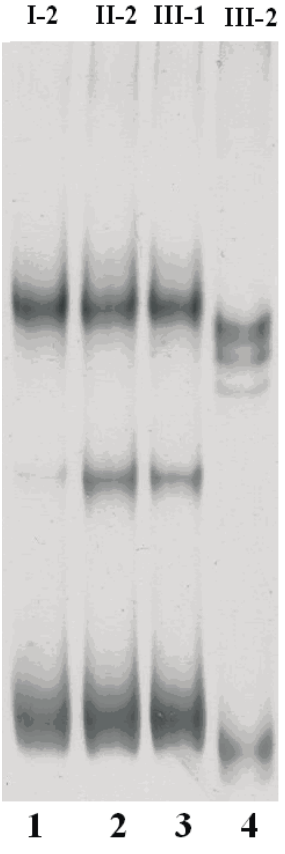

Figure 2. SSCP analysis of *APC* gene, exon 15G. Positive members of the family analyzed are in lines 1 (proband´s grandfather, I-2), 2 (proband´s mother, II-2) and 3 (proband, III-1). In comparison, the negative member in line 4 (proband´s brother, III-2) is without extra bands.

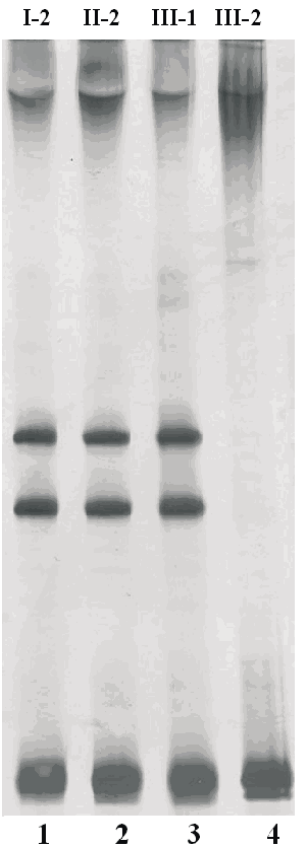

Figure 3. Heteroduplex analysis of the family analyzed. Formation of heteroduplex is seen in lines 1 (proband´s grandfather, I-2), 2 (proband´s mother, II-2) and 3 (proband, III-1). Line 4 (proband´s brother, III-2) represents negative control.

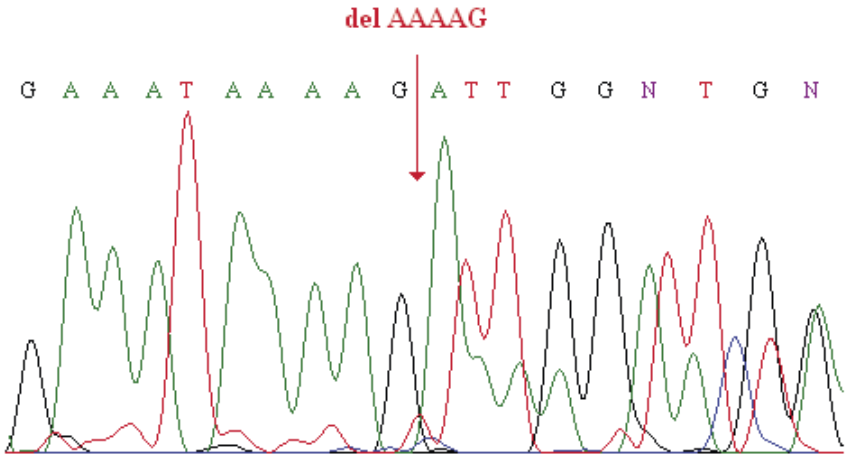

Figure 4. Sequencing of *APC* gene, exon 15G, positive member (III-1) of the family analyzed with 5 base pair deletion at codon 1309.

Detail analysis of expected mutations in segment 15 G was performed by automatic sequencing. The 5 base pair deletion at codon 1309 (3927_3931delAAAGA) was identified by direct sequencing (Fig.4).

Discussion

The risk of developing specific manifestations of FAP is often correlated with the position of the inherited *APC* mutation. The attenuated phenotype (<100 colorectal polyps) is restricted to mutations before codon 157, after codon 1595 and in the alternatively spliced region of exon 9 [9, 15]. A profuse phenotype (thousands of polyps) is associated with mutations from codons 1250 to 1464 [16]. The occurrence of CHRPE seems to be restricted to codons 311-1444 [17] and desmoid tumors appear to be limited to patients with mutations between codons 1403 and 1578 [18].

In three members of the family studied – proband, his mother and grandfather, we identified the same mutation, deletion of 5 base pair (AAAGA) at codon 1309. This mutation causes a frameshift in translation and termination occurs at codon 1313. It is the most frequent mutation and its frequency varies from population to population. In Slovakia this mutation represents approximately 15% of all identified mutations [18], which is comparable to frequencies reported from Italy [17] and Singapore [20].

As regards aggressivity of the disease, mutations at codon 1309 have been typically associated with a more severe clinical phenotype [21]. Patients with mutations at this site tend to develop bowel symptoms more than 10 years earlier (mean 19.8 years) than those with mutations at other sites [22], and have significantly more colorectal polyps. Mutations at codon 1309 are also associated with an earlier age of CRC development (mean 35 years). An asymptomatic patient has never been reported having this mutation.

In this report we describe three members of one family with the same mutation and very different phenotype. Our proband was a young man with very early onset of the disease – at the age of 18. Polyps in the large bowel were identified at the age of 14. This phenotype is typical in a patient with mutation at codon 1309. Clinical information about the proband´s mother is not available, but we identified the same mutation in his grandfather, who was asymptomatic. He is exceptional in that he had *APC* mutation and did not develop cancer. A very similar case was reported by Vaynsthein [23] concerning one patient with Gardner's syndrome. This patient had FAP for 40 years and she did not develop cancer. It is possible that some individuals with APC mutation have a benign course and are probably never diagnosed due to the absence of any symptoms. Gebert with coworkers [24] reported one patient with a deletion at codon 1309, who was diagnosed at the age of 42, whereas one of his affected sons became symptomatic at the age of 7. Thus a sequence alteration at codon 1309 of exon 15 does not necessarily result in a phenotype with an early disease onset.

One should keep in mind that the site of mutation will not exactly predict a certain gene product and a subsequent phenotype. It is likely that additional individual genetic and environmental factors may play important roles in determining the colonic as well as the extracolonic manifestations [25].

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