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Familial polyposis coli – inducing mutations in APC gene in Poland

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Abstract. Screening for molecular changes within the adenomatous polyposis coli (APC) gene, exons 11-14 and the 5' half of exon 15, encompassing the mutation cluster region within exon 15, was performed in 30 patients with Familial Polyposis Coli (FAP). All patients were studied by heteroduplex analysis (HA) and single strand conformation polymorphism (SSCP) and molecular changes were found in 7 cases. Protein truncation test (PTT) has been performed in 17 cases in which mutations have not been found earlier, and shortening of protein product was noted in 2 cases. In three cases common deletion of 5 bp at codon 1309 and in one 5 bp deletion at codon 1061 were found. In other cases the molecular changes were demonstrated as heteroduplexes in exon 14 (1 patient), in segments E and F (one patient each) of exon 15, and in two cases the heteroduplexes were within the overlapping sequences of segments E/F and F/G of exon 15, respectively. In families where the molecular changes were found by HA, 7 persons at high risk for FAP were found and advised to undergo regular endoscopic examinations. In three persons at risk the transfer of mutation was excluded.

Key words: adenomatous polyposis coli, polymerase chain reaction, single stranded conformational polymorphism, heteroduplex analysis, protein truncation test, polish population.

Introduction

Familial adenomatous polyposis coli (FAP) is a dominantly inherited autosomal disorder characterized by early onset of multiple adenomatous polyps

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in colon and rectum, that lead to development of colorectal carcinoma. FAP is caused by germ line mutation in the gene localized on chromosome 5 in region q21 (BODMER et al. 1987).

Among hereditary colon cancers FAP is the second in frequency to hereditary nonpolyposis colon cancer (HNPCC). A distinct feature of FAP as compared to HNPCC and most other forms of hereditary cancer is development of multiple polyps in colonic mucosa preceeding the malignant transformation by years. That has made possible the diagnosis of the FAP-inducing mutations and related susceptibility to colon cancer in a period before advent of molecular studies. In Finland HNPCC makes up about 2% of all colon cancer cases, whereas the frequency of FAP is severalfold smaller (MECKLIN et al. 1995). In Denmark FAP patients constituted 0.07% of all Danish patients with colorectal cancer in years 1980-1992. This low relative percentage was found in spite of the prevalence of FAP as high as 32×10^{-6} at the end of 1992 (BÜLOW et al. 1996).

The gene mutated in FAP was named Adenomatous Polyposis Coli (APC). The other symptoms caused by the mutations of APC gene, are gastrointestinal polyps osteomata of jaw, sebaceous cysts and pigmented retinal lesions. In Mc KUSSICK's catalogue of mendelian inheritance in man (1994) the APC gene mutations are listed under the heading of Adenomateous Intestinal Polyposis (MIM 175100). Polyps and cancer in FAP patients are not confined to colon and occur relatively frequently in duodenum (BÜLOW et al. 1995). The cDNA sequence of APC gene was described for the first time by GRODEN et al. in 1991. The APC gene consists of fifteen exons, total length of 8532 bp and codes protein of 2844 aminoacids (GRODEN et al. 1995). Recently an additional exon localized between exons 10 and 11 and named 10A has been described (SULEKOVA et al. 1995). The development of colon carcinoma in FAP patients is associated with a loss of normal allele of APC gene (loss of heterozygosity). Furthermore, introduction of APC gene by transfection into human colon carcinoma cell lines suppressed tumorigenicity of these cells in nude mice (GRODEN et al. 1995). This proves that APC gene is a tumor suppressor gene. Accumulation of mutations in a number of other tumor suppressor genes is a prerequisite for malignant transformation. The APC gene is expressed in most of the tissues, but only some of them, such as duodenum, pancreas and stomach are susceptible to malignant transformation in consequence of its mutation.

The APC protein is approximately 300 kD weight, and located in cytoplasm. The associations of the APC gene product with the catenins and (E-cadhedrin-associated proteins) suggest that it played its role in the maintenance of cell to cell interactions (SU et al. 1993).

Most of the mutations causing FAP are clustered at the 5' half of exon 15. Common types of the FAP-inducing APC mutations are deletions and insertions, which produce reading frameshifts, generating stop codons and, consequently, truncation of the gene product (BEROUD, SOUSSI 1996).

In the present study the heteroduplex analysis (HA) and single strand conformation polymorphism (SSCP) tests were used to detect mutations in the studied parts of APC gene in FAP patients. In the patients in which molecular change was not defined by this approach the protein truncation test (PTT) was used as the method specific of the FAP-inducing APC mutations (PAWLAK et al. 1996).

Material and methods

The blood samples were obtained from FAP patients and their families. Genomic DNA was prepared using standard methods and DNA samples were stored at –20°C.

The FAP patients from each family were screened with HA and SSCP tests for the most common mutations in APC gene. The region encompassing nucleotides 3082-4125 of the APC gene was amplified with the use of three pairs of primers according to Groden 15E, 15F and 15G. The exons 11, 12, 13, 14 were also screened.

The PCR products were obtained by standard PCR conditions. The PCR products were tested by the heteroduplex analysis (HA) on 10% nondenaturing polyacrylamide gel containing 10% glycerol. In SSCP test, after heat denaturation of DNA in 80% formamide, electrophoresis was performed on 5% nondenaturing polyacrylamide gel containing 5% glycerol. In both HA and SSCP tests the products were visualized by silver staining (FRIEDL et al. 1993).

The PCR product for PTT analysis was obtained by standard PCR using PTT forward primer (van der LUIJT et al. 1994) comprising the phage T7 promoter sequence, followed by a Kozak translation sequence, an AUG translation start signal and an in frame gene specific sequence. The reverse primer should be designed to generate a PCR product of up to 2000 bp. The resulting PCR product was used as DNA template for transcription. The transcription and translation with L-[^{35}S] methionine incorporation was performed using TNT coupled reticulocyte lysate system (Promega Corporation Madison WI, USA). The final protein was separated on 12.5% SDS polyacrylamide gel. The gel was fixed in 10% methanol and 20% acetic acid solution for 10 min. and dried on gel dryer at 80°C for 2 hours. The autoradiography was performed on Kodak X-mat film for 48h at room temp. (HAMZEHLOEI, WEST 1996).

The primers for the genomic DNA PTT of segment A, exon 15 of APC gene, according to van der LUIJT et al. (1994) (exon 15 E-J, codons 1028-1701) were synthetized by R. Adamiak from the Institute of Bioorganic Chemistry Polish Academy of Sciences. The other primers for the genomic DNA PTT test of Exon 15 of APC gene: segment 2 (exon 15 A-F, codons 658-1283) and segment 3 (exon 15 E-J, codons 1097-1701) were developed in the laboratory of S. West.

Results

Mutations of APC gene were analysed in 30 families with familial adenomatous polyposis (FAP) coli. So far, exons 11-14, and the 5 half of exon 15, encompassing codons 470-1639 of the APC gene were screened for mutations by the use of heteroduplex and SSCP methods. The molecular changes noted in the studied series of patients are summarized in Table 1. The 5 bp deletion

Table 1. Molecular changes in the studied series of FAP patients

Patient	Molecular change	Phenotype
A. Mutations in exon 15 APC gene		
F1	AAAGA, codon 1309	severe
F11	AAAGA, codon 1309	severe
F13	AAAGA, codon 1309	severe
F41	ACAAA, codon 1061	average
B. Heteroduplex positive cases; number of exon and segment of exon 15 indicated		
F26	15E and 15F	severe, thyroid ca
F32	15F and 15G	severe
F3	14	average
F41	15E	average
F30	15F	no data
C. Cases showing truncation of APC protein		
F43	segment A of exon 15	average
F34	segment 3 of exon 15	average

at codon 1309 was identified in 3 families and a 5 bp deletion at codon 1061 in one case. In addition, aberrant heteroduplex patterns were observed in three

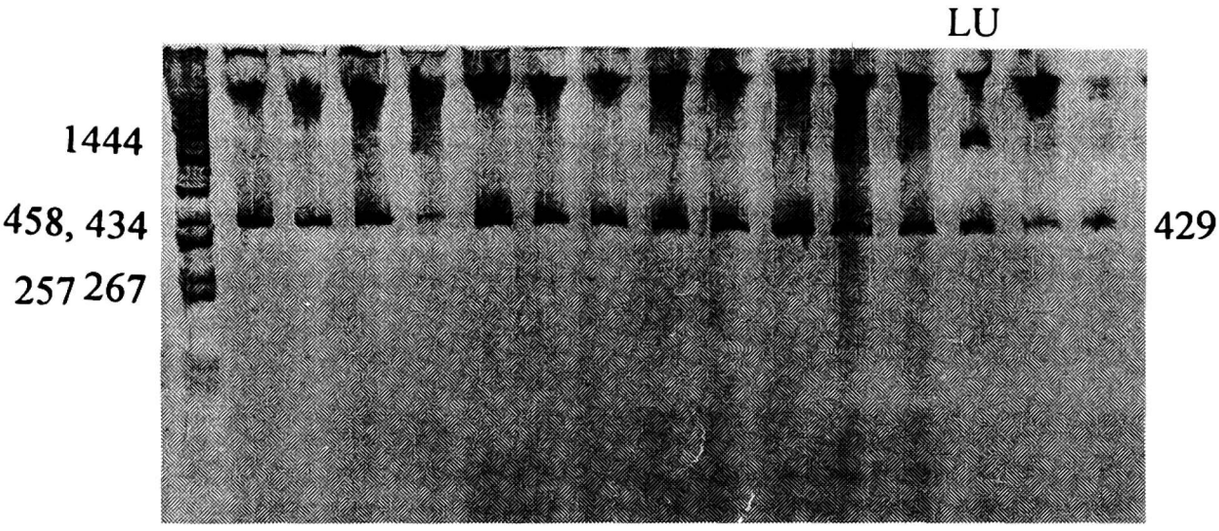

Fig. 1. Heteroduplex analysis of segment F of APC gene from 18 patients with FAP
Marker DNA was from pUC19 plasmid digested with HaeIII and TaqI. Lane 13 – heteroduplex present in patient LU

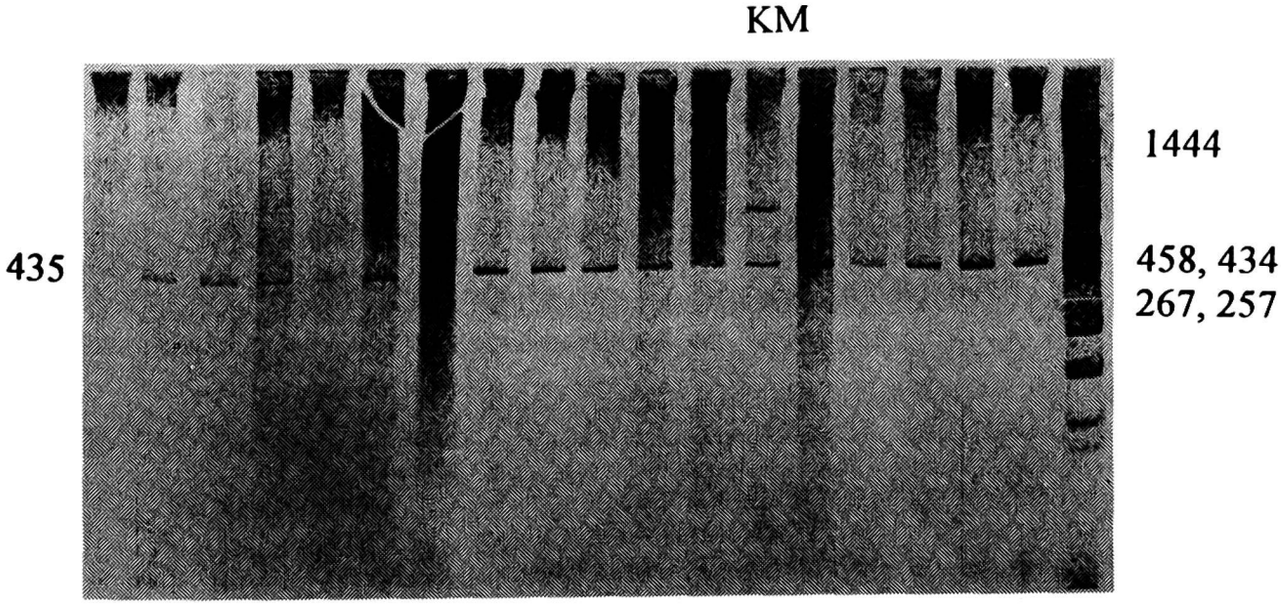

Fig. 2. Heteroduplex analysis of segment E of APC gene from 15 patients with FAP
Marker DNA was from pUC19 plasmid digested with HaeIII and TaqI. Lane 14 – heteroduplex present in patient KM

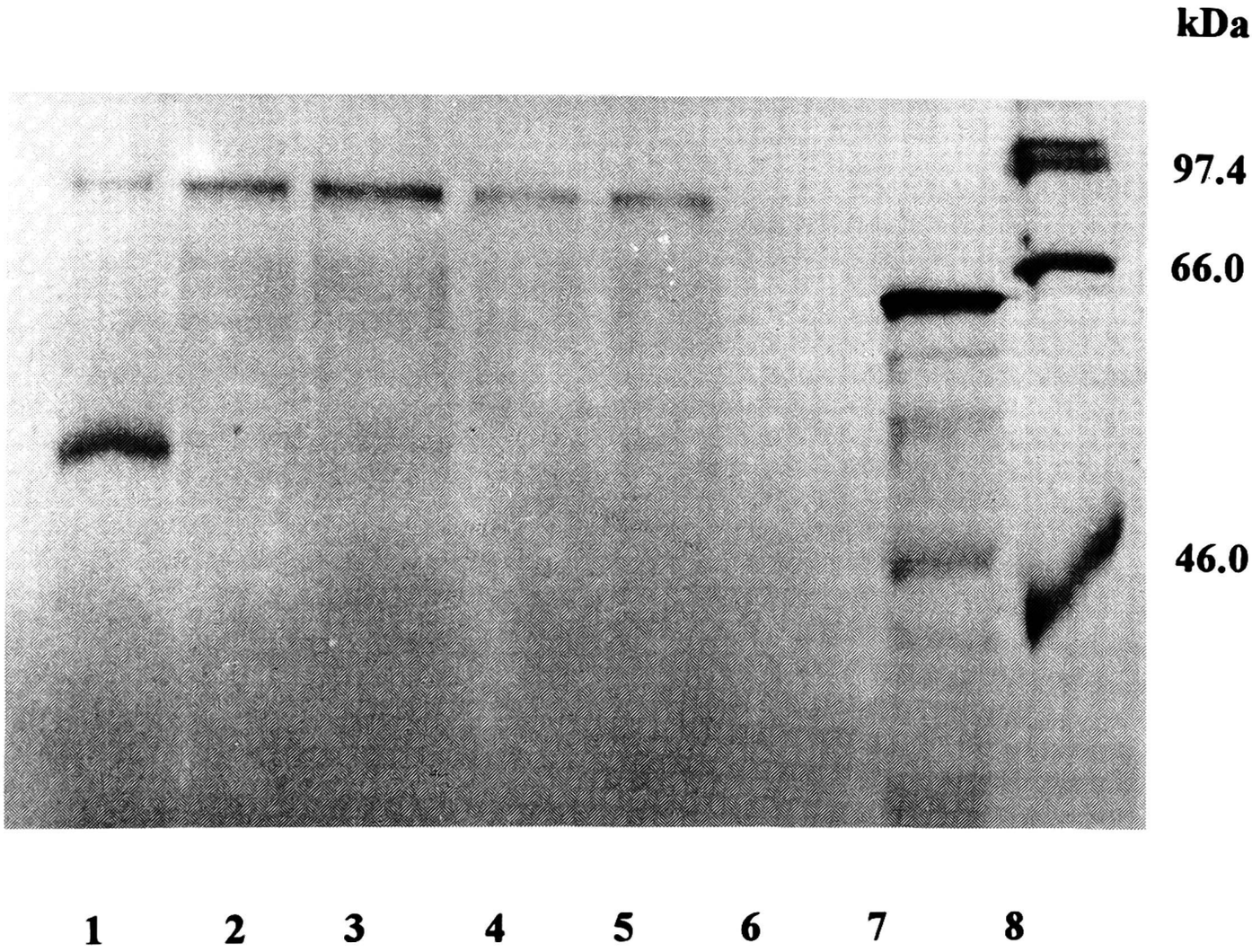

Fig. 3. Protein truncation test with primers for segment 3 exon 15 of APC gene (codons 1097-1701)

Lane 1 – patient F34, mutation demonstrated, lanes 2-5 – patients F21, F22, F30 and F32 – normal product, lane 6 – no DNA, lane 7 – luciferase control, lane 8 – Rainbow marker

other FAP families, in exon 14 and in 15, and on the border of segments E and F (1) as well as in overlapping region of segments F and G (Figs. 1, 2).

From the pedigree analysis the high risk of disease was recognized in 17 persons from 6 families in which molecular changes were defined by HA and 10 of them were studied. Presence of mutation was confirmed in 7 of the studied persons; in 4 of them the diagnosis of mutation was presymptomatic. Persons at high risk for FAP were advised to undergo regular endoscopic examinations. In three persons, including a 10 y old boy, the transfer of mutation was excluded.

17 patients who did not show molecular changes with HA and SSCP methods, were studied for protein product of the segment of exon 15, codons 658-1701. Premature termination of translation (PTT) was found in 2 of them (Fig. 3, Table 1). In total, the molecular changes in APC gene were found in 10 patients (Table 1).

Discussion

The Polish registry of familial polyposis coli dates from 1980 and comprises some 70 families (KROKOWICZ 1990), but up to now only 30 of them were analysed for molecular changes in APC gene.

Diagnosis of new mutations in APC gene usually starts with relatively simple screening methods such as the denaturing gradient gel electrophoresis (DGGE) (FODDE et al. 1992) and HA (FRIEDL et al. 1993, HAMZEHLOEI et al. 1994). In our study HA was performed in all cases and the changes were noted in 7 of 30.

The in relative effectiveness of heteroduplex analysis (HA) and single strand conformation polymorphism (SSCP) methods used by us for visualization of the mutation differed, depending on the localization of the mutation within the PCR-product. The peripheral localization of the mutation in the segments F and G of exon 15, as evidenced by the finding of heteroduplexes in both segments, was found to be an obstacle in demonstrating the mutation with SSCP (data not shown). This indicates that HA and SSCP, the methods used by many laboratories to study FAP patients, display complementarity one to another, in particular in cases of mutations located at the border of the segments of DNA analysed by PCR.

The most frequent single types of APC gene mutation: the 5 bp deletion at codon 1061 and 5 bp deletion at codon 1309 account for approximately 15 percent of all germline mutations in FAP patients and belong to a class of the FAP-inducing mutations causing truncation of the gene product.

The risk of developing cancer in persons with the FAP-inducing inherited mutation in APC gene is close to 100%. The silent variants of APC gene have, however, to be differentiated from pathogenic, FAP-inducing mutations (POWELL et al. 1992, BEROUD, SOUSSI 1996).

The 1309 del mutation occurs in the studied families with the same frequency (3/30 = 10%) as that reported in the other groups of FAP patients (MIYOSHI et al. 1992, MANDL et al. 1994, MIYAKI et al. 1994). The age of cancer occurrence varies depending on the type and location of mutation. The most common mutation, 5 bp deletion at codon 1309, is considered to be a relatively more severe defect with the earlier onset of cancer as compared to the rest of FAP cases (CASPARI et al. 1994). On the other hand, in different families and in individual cases the age of malignant transformation varies considerably (CAMA et al. 1995).

In genetic diseases caused by nonsense and frameshifting mutations, the premature termination of transcription occurs as a measurable event. As a basis for a diagnostic test the premature termination of transcription (PTT) has been postulated for the first time in Duchenne Muscular Dystrophy (DMD). Aiming at detection in one PTT test of mutations in several exons of DMD gene, the test was performed on cDNA (ROERST et al. 1993). APC gene mutations predominantly occur within the large exon 15, comprising 6580 bp., that is 77% of the gene sequence, which contains all the known mutation hotspots. For this reason measurements of the premature termination of transcription of the exon 15 of APC gene in genomic DNA may contribute to the diagnosis of a number of FAP-inducing mutations (van der LUIJT et al. 1994).

The possibility that such factors as digestion-resistant starch in diet and aspirin treatment, may delay the development of polyps in colon were considered on the basis of clinical observations. Now the effectiveness of such procedures to slow down the growth of polyps can be assessed in the controlled trial *in vivo* by observation of persons with molecular diagnosis of germinal mutations in APC gene at young age before development of polyps (BURN et al. 1995).

The reported progress in molecular diagnosis of APC gene mutations as well as prospects of effective control over its expression should also be considered of importance because of the frequent occurrence of somatic mutations within this gene. On the basis of the incidence of adenomatous polyps in the general population (RANSOHOFF, LANG 1991) and the somatic mutation rate of the APC gene it may be assumed that these mutations lead to benign colorectal tumors in about one-third of the population of the Western World. If not surgically removed, 15-20% of these polyps will progress to cancer (SU

et al. 1993). Presumably the dietetic and pharmacologic treatments devised for slowing down growth of polyps in the hereditary APC gene deficiency will also be applicable for delaying the development of polyps produced by somatic mutations of this gene.

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