ORIGINAL ARTICLE

Hereditary Colorectal Adenomatous Polyposis Syndromes

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ABSTRACT

Kohoutová M., Štekrová J., Šulová M. et al.: Hereditary Colorectal Adenomatous Polyposis Syndromes
Background: Hereditary colorectal adenomatous polyposis syndromes are a predisposition to colorectal carcinoma development. The familial adenomatous polyposis is the most common analyzed syndrome that results from germ-line mutations in the APC gene. In addition, the autosomal recessive form of polyposis has recently been reported. This disease is caused by germ-line mutations in the base excision repair MYH gene. The goal of this study is the identification of genetic causes of the colorectal polyposis, the determination of the frequency and type of the APC and MYH germ-line mutations in the set of families with colorectal polyposis in the Czech population.
Methods and Results: The set of 103 probands with FAP was screened for germ-line APC mutations using the Protein Truncation Test and Denaturing Gradient Gel Electrophoresis. MYH mutational screening was performed on 60 unrelated patients without detected APC mutations using the Denaturing High Performance Liquid Chromatography. Automated sequencing was carried out to identify mutations found. In total the 51 germ-line APC mutations (69.9 %) are reported in the set of 72 probands including 31 novel mutations unique for the Czech population. Molecular genetic analysis of the MYH gene revealed 15 DNA variations (25 %) including two patients identified as p.Y165C/p.G382D compound heterozygotes (3.3 %) and 13 polymorphisms or intronic changes (21.7 %). The novel variants were detected in 5 patients.
Conclusion: The present study reflects the extremely heterogeneous spectrum of the APC mutations in the Czech population and confirms the previously reported data. However, the changes found in the MYH gene still need more extensive studies. Our results are important for genetic counselling and further clinical management among at-risk family members. It also enables distinction between different types of the colorectal polyposis.
Key words: familial adenomatous polyposis, APC gene, MYH gene, mutational analysis. *Ko.*

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The occurrence of colorectal adenomatous polyposis can have sporadic, familial or hereditary character. In any case, it represents a risk of developing colorectal cancer (CRC). Hereditary colorectal cancer appears in approximately 20 % of all cases. Among the hereditary CRC, familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) are the most explored syndromes. Research of these syndromes has provided identification and description of the genetic features which are associated with colorectal carcinogenesis not only in hereditary but also in sporadic forms of the disease. Knowledge of the disease's molecular genetic basis permits to perform presymptomatic diagnostics on the DNA level and to develop a preventive and treatment strategy.

Familial adenomatous polyposis (FAP, MIM # 175100) is generally regarded as an autosomal dominant inherited predisposition for the development of CRC. The incidence of the disease is estimated at 1 per 5000 to 7500 individuals in the Czech population. The frequency of the disease is often associated with mutations *de novo.* FAP appears in two forms. Classical FAP is characterized by hundreds to thousands of adenomatous polyps in the large intestine and rectum with the early onset of the disease (in the 2nd decade of life). In a large amount of the polyps, some of them develop adenocarcinomas gradually. The disease is also associated with a number of extracolic manifestations (1) such as congenital hypertrophy of the retinal pigment epithelium (CHRPE), the occurrence of polyps in duodenum, the occurrence of desmoid tumours, epidermoid cysts and osteomas. The occurrence of hepatoblastoma is also not rare. CHRPE appears in approximately 80 % of patients and therefore it is an important diagnostic factor. Its occurrence depends on a localisation of germ-line mutation in the *APC* gene but the absence of it does not exclude the FAP disease. Attenuated FAP (AFAP) is characterized by less number of polyps (usually under 100) and by the later onset of the disease. However, the risk of developing CRC is also high in AFAP.

The FAP disease is caused by germline mutations in the *APC* (adenomatous polyposis coli) gene. Although the variability in phenotype is considerable, the disease is very penetrating and develops in practically 100 % of the mutation carriers. In families where FAP occurs it is necessary to perform the diagnosis in individuals at risk in time. The risk of the disease's development is 50 % in a descendant of an affected individual. Following medical

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help can be performed to prevent CRC development. Somatic mutations in the *APC* gene have been identified in approximately 80 % of sporadic CRC. In both cases the *APC* mutations play a very important role in the initiation of multistep process of colorectal carcinogenesis.

The *APC* gene is a tumour suppressor gene which is located in chromosome 5(q21) (2) and identified by a position cloning of FAP locus (3, 4). The coding gene sequence consists of 15 exons, and the exon 15 is the largest known exon in the human genome to date. The mutations in both alleles are necessary for inactivation of the gene. In the case of hereditary colorectal cancer one of the *APC* mutations is inherited from one of parents (germ-line mutation). The second allele is mutated in epithelial bowel cells (somatic mutation). Both alleles of the gene are mutated at the somatic level in the sporadic CRC. In the *APC* gene more than 1400 germ-line and somatic mutations have been reported to date leading to the non-functional protein product (5). The *APC* gene codes APC protein, which is present in a variety of tissue cells. This protein is a part of the very old signalling pathway (Wnt pathway). Its key role is a regulation of ß-catenin level in cytoplasm. As a result of inactivation of APC protein, ß-catenin is accumulated in cytoplasm, and then it is transported into a nucleus. In this place it activates a transcription of target genes (protoonkogene c-myc, cyclin D1 etc.) together with other factors (TCF – T cell factors, LEF – lymphoid enhancer factor) and regulates the course of the cell cycle. The APC protein takes part in a regulation of the proliferation, differentiation, migration and apoptosis of cells. APC also plays a role in controlling the cell cycle, stabilizes microtubules and controls segregation of chromosomes (6).

FAP AND HETEROGENEITY

Over-intensive mutational analysis, germ-line mutations in the *APC* gene are detected in 60-80 % of families with FAP. Using standard methods of mutation analysis a molecular genetic cause is not detected in approximately 20-40 % of families with FAP. The explanation may lie in the existence of large sub-microscopic deletions of the whole gene or its parts (7,8) which cannot be screened by standard methods of mutational analysis, or in mutations of the regulation gene region. However, an occurrence of mutations in other genes cannot be eliminated.

The possibility of the occurrence of other genes within a disease's development has been recently supported by findings in a group of patients with multiple adenomas. The term "multiple adenomas" means that number of polyps varies between 3 and 100 in the colon and rectum and it is much lower than in classical FAP. Patients have a milder phenotype. They are often the only ones who may be affected in families, and germ-line *APC* mutation (7) does not have to be detected in them. In this group of the patients it is necessary to consider AFAP or HNPCC during differential diagnostics or a new hereditary form of polyposis which has been identified recently. In some patients, recent studies have revealed a disorder in the base excision repair system of the DNA. It is related to germ-line mutations in the *MYH* gene (MutY homolog *Escherichia coli*) which cause the autosomal recessive (AR) pattern of polyposis (9-11). The *MYH* gene maps to the short arm of chromosome 1p34.3–1p32.1 and its product is a component of the base excision repair machinery (BER – base excision repair). This system plays an important role in the repair of mutations causing by oxidative products which develop during aerobic metabolism. The most stable product of oxidative DNA damage is 8-oxoG (8-oxo-7,8,-dihydroxy2'deoxyguanosine) which mispairs with adenine instead of cytosine leading to G:C→T:A transversion mutations. Analysis of the *APC* somatic mutation spectrum revealed high frequency of G:C→T:A transversion in tumour samples. This explains the occurrence of symptoms which are similar to FAP or AFAP. The *MYH* gene product is adenine specific DNA glycosylase which removes adenine mispaired with G or 8-oxoG. Two other enzymes, MTH1 and OGG1 are part of the base excision repair system. They work together and prevent mutagenesis inducing by 8-oxoG.

For these reasons the molecular genetic testing of individuals with both the large or small numbers of colorectal adenomatous polyps is very important. AR pattern of inheritance explains the occurrence of diseases in a group of patients with negative family history. It is conditioned by finding of a biallelic *MYH* germ-line mutations. Differential diagnostic importance of the molecular genetic tests is essential to ensure timely presymptomatic DNA diagnosis of individuals at risk and to provide suitable preventive interventions.

The aim of the work is to establish genetic causes of colorectal polyposis, to determine the frequency and type of *APC* and *MYH* germline mutations in a set of Czech FAP families and to provide presymptomatic DNA diagnostics in relatives who have not yet been clinically affected.

PATIENTS AND USED METHODS

Screening of APC germ-line mutations

Patients: The set of patients consists of 180 families with colorectal polyposis. One hundred and three unrelated probands with FAP have been included in a study. Patients who come from different medical institutions in the Czech Republic are recommended for an examination on the basis of colonoscopic findings or positive family history. Before genetic tests they sign written informed consent with a molecular genetic examination.

Methods: DNA was isolated from peripheral blood lymphocytes (12) and amplified by polymerase chain reaction (PCR) using series of overlapping oligonucleotide primers which are enriched by GC sequences. The examination covers the whole *APC* gene. Then mutational analysis by DGGE method (**D**enaturing **G**radient **G**el **E**lectrophoresis) (13, 14) and by PTT (**P**rotein **T**runcation **T**est) (15) followed. The last step was sequence analysis using the automatic ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystem).

Screening of MYH germline mutations

Patients: Sixty unrelated patients have been included in the study. In these patients germ-line mutations in the *APC* gene were not detected by classical methods. In this group 34 patients with multiple colorectal adenomas (3-100) and 26 patients with classical FAP (>100 polyps) were examined.

Methods: DNA was isolated from peripheral blood lymphocytes (12) and amplified by polymerase chain reaction (PCR). Mutational analysis was performed by DHPLC method (Denaturing High Performance Liquid Chromatography) with system WAVE (transgenomic). The type and exact localization of mutation were defined by a following sequence analysis on the automatic ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystem).

RESULTS

In the set of 103 probands a total of 51 *APC* germ-line mutations were identified in 72 probands. This is approximately 70 % capture of mutations (69.9%). In addition, 31 novel mutations unique for the Czech population were detected in the group of 33 probands. Twenty mutations detected in the group of 39 probands were

reported by other authors. Mutations were detected mostly in the 5'end of the *APC* gene: 30 mutations (41.7 %) were detected in exons 1-14 and 42 mutations (58.3 %) in exon 15. The most frequent mutations found were deletions of 5 bp at codon 1309 detected in 7 cases (9.7 %) and deletions of 5 bp at codon 1061 found in 3 cases (4.2 %). Relatively higher frequency of mutations was located at codon 935 (5.5 %), 541 (4.2 %) and 213 (4.2 %). Predictive tests were performed in 188 relatives at risk. Mutation was confirmed in 74 cases and eliminated in 114 individuals.

The importance of molecular genetic tests in differential diagnostics results can be demonstrated in an example of one family examined in our laboratory (16). A proband was examined due to small pains in the right hypogastrium at the age of 20. A resistance was detected by the examination in coecum and colon ascendent. The diagnosis of coecum cancer was confirmed, and then right side hemicolectomy was performed. The proband was recommended for a molecular genetic testing of HNPCC genes with regard to the early onset of the disease and right side localization of cancer. The family history did not reveal any hereditary colorectal cancer. During the examination the patient was the first affected person in the family (Fig. 1). The analysis of the *MLH1* and *MSH2* genes, whose germ-line mutations cause HNPCC, did not reveal any changes in the DNA sequence. Subsequent report from a pathologist informed us that sporadic small polyps were detected in bowel resection and on this basis mutational analysis of the *APC* gene was performed. The result was the identification of germ-line substitution at codon 213 (exon 5) (c.637C>T) (Fig.2, 3) leading to a stop codon and occurrence of a short non-functional APC protein. After this finding

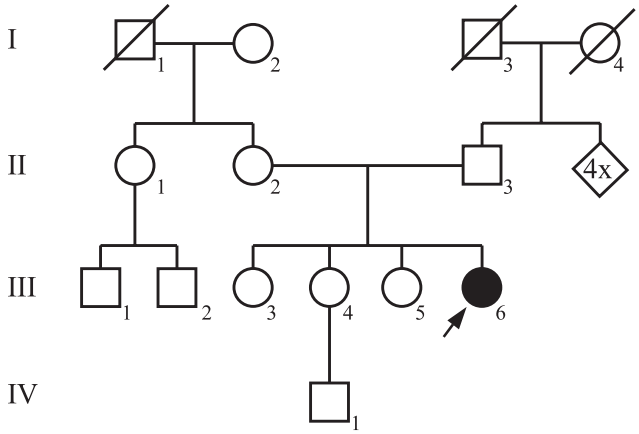

Fig. 1. Family pedigree before molecular genetic testing. The proband (III/6) (coecum cancer) is only affected in the family.

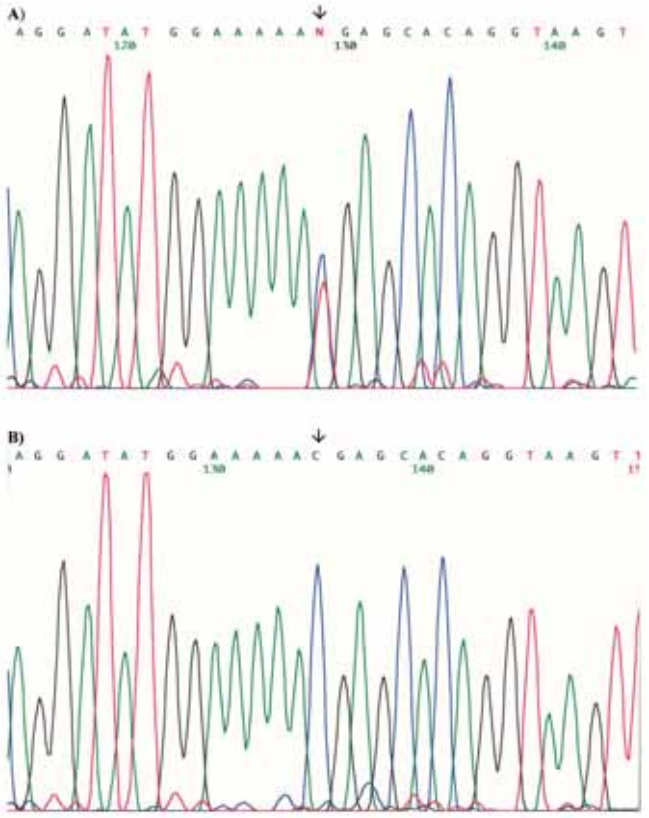

Fig. 3. Identification of the mutation by sequencing
A. The proband III/6 with established mutation (arrow) c.637CT (p.Arg213X) leading to the creation of the stop codon and of the shortened APC protein.
B. The son (IV/1) of the proband's affected sister who is at risk of 50 %. He is excluded as the carrier of the mutation.

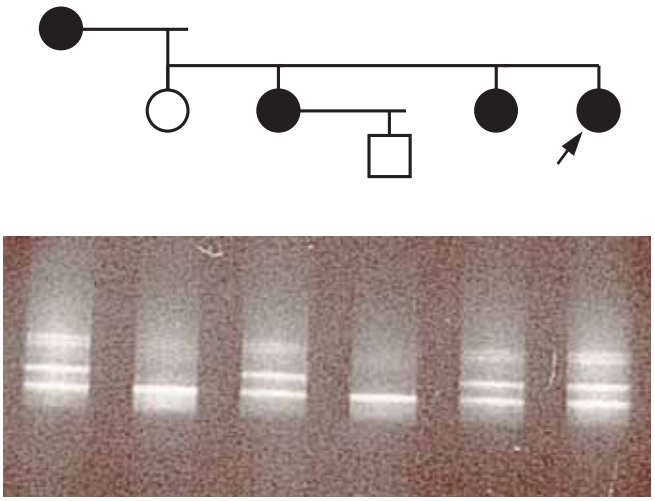

Fig. 2. Examination of family members by DGGE method
The mutation was detected in the mother (II/2) and two sisters (III/4, III/5) of the proband (III/6). The third sister (III/3) and the son (IV/1) of one affected sister are not carriers of the mutation.

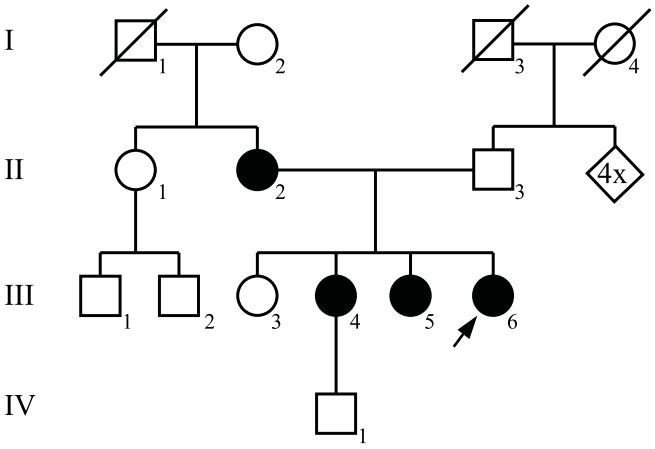

Fig. 4. Family pedigree after molecular genetic testing of the proband and her relatives.
Carriers of the mutation are the proband (III/6), her mother (II/2) and two sisters (III/4, III/5). The mutation was excluded in proband's sister (III/3) and in the son (IV/1) of the affected sister (III/4). The results are in accordance with findings of colonoscopic examination.

the DNA analysis was carry out in relatives of the proband (mother II/2, sisters III/3, III/4, III/5 and son IV/1) with these results (Fig. 4, p. 478): The causal mutation was confirmed in the sisters III/4 (27 years old), III/5 (23 years old) and in the mother II/2 (49 years old) and eliminated in the sister III/3 and the son IV/1 (6 years old) of the proband's affected sister. The follow-up colonoscopic examinations of relatives confirmed the results of the molecular tests.

The *MYH* mutation analysis revealed 15 variations of the DNA sequence (25 %) in the set of 60 probands. Two of the patients (3.3 %) were compound heterozygotes for the most frequent known variations p.Y165C and p.G382D which cause colorectal polyposis. Phenotypes of these patients corresponded to AFAP. Neither CRC, nor multiple adenomas were found in their family history. In addition, 13 polymorphisms and/or intronic changes (21.7 %) were detected. Five of them were novel genetic alterations (8.3 %) localized mainly in the *MYH* introns.

DISCUSSION

This work is the result of over 12 years of studying the mutational spectrum in the Czech patients affected by FAP, AFAP or with an occurrence of multiple adenomas (17, 18). It confirms the extremely different spectrum of germ-line mutations in the *APC* gene. Routine methods of mutation screening are not successful in approximately 30 % of patients with classical FAP and in 90 % of patients with AFAP/multiple adenomas. This can be caused by large submicroscopic deletions of the *APC* gene, by existence of mutations which influence the expression of allele or by mutations in the other genes. The spectrum of the Czech *APC* mutations is no different from reported data. In the Czech population there is also large phenotype variability not just in unrelated probands, but also in individuals with identical mutation even within the one family. These results also confirm that the resultant phenotype can be influenced by factors of the external and internal environment, including the possibility of influence of modifying genes from the genetic background.

The importance of this work lies in the possibility of timely presymptomatic diagnostics of disease in individuals at risk and of differential DNA diagnostics among individual variants of hereditary colorectal polyposis. Carriers of germ-line mutations in these genes have been regularly monitored and subjected to colonoscopic examination in gastroenterology departments, and on the basis of clinical picture preventive interventions (colectomy) can be performed against development of CRC. Individuals without pathological allele can be removed from this programme. Despite of the possibility to provide presymptomatic testing and to know the genotype – phenotype correlation, the high variability of phenotypes does not allow the prediction of specific prognosis of the disease.

This work confirms recent information that biallelic germ-line mutations in the *MYH* gene can cause the phenotype of multiple adenomas and that this form of polyposis is inherited by the autosomal recessive pattern and can be considered attenuated FAP. Similar considerations can be noted in connection with the occurrence of the familial and sporadic CRC whereas undisclosed genes cannot be excluded. Now it is too early to consider the importance of other new or old changes in the *MYH* gene. The more detailed analysis of *MYH* variants and of clinical correlation is required. Nevertheless, they are an important part of presymptomatic diagnostics at present time.

Besides the practical results, the works concerning in the molecular analysis of the hereditary CRC have a considerable importance for understanding a mechanism of colorectal carcinogenesis in sporadic CRC. Currently, several pathways of colorectal carcinogenesis are considered:

1) Somatic mutations in the *APC* gene (approximately 80 % of sporadic CRC) initiate a multistep process of carcinogenesis. They are followed by mutations in protooncogene *K-ras,* by allelic loss of a region of the chromosome 18q, by mutations in *SMAD4* gene in some cases and by mutations in *p53* gene. These cancers are characterized by chromosomal instability – CIN + (chromosomal instability) because an aneuploid or polyploid karyotype is found in cells. (19).

2) The *MMR* (Mismatch Repair genes) mutations are responsible for approximately 15 % of sporadic CRC development. The products of the MMR genes repair incorrect placed nucleotides during DNA replication. These cancers are noted for instability in the DNA repetitive sequences calling microsatellites (MSI – microsatellite instability). They are MSI-H (high). They are not characterized by a chromosomal instability (CIN-) because their karyotype is almost diploid. A high methylation of CpG islands in the promoter region of the *MMR* gene is often the cause of the inhibition of a gene transcription (20).

3) A third group of CRCs does not show microsatellite instability or MSI is very low – tumours are MSS (microsatellite stable) or MSI-L (low). In some publications it is discussed that this CRCs form a separate group with specific molecular features, but other data disclaim this (20).

4) It seems that MAP (*MYH* associated polyposis) (19) carcinogenesis is different from the previous ones but has some similar features which are characteristic for both the previous groups. Tumours show high frequency of mutations in the *APC* gene, relatively high frequency of mutations in *K-ras* genes and loss of heterozygosity in the region of 18q like tumours of the first group (CIN+). However, they are practically diploid (CIN-) like tumours of the second group (MSI+).

Hypothetically, it seems that colorectal cancers have always one type of instability: CIN, MSI or BER. A confirmation of this idea requires other more thorough analysis.

Abbreviations:

APC	– adenomatous polyposis coli
AFAP	– attenuated familial adenomatous polyposis
BER	– base excision repair
CHRPE	– congenital hypertrophy of the retinal pigmental epithelium
CIN	– chromosomal instability
CRC	– colorectal cancer
DGGE	– denaturing gradient gel electrophoresis
DHPLC	– denaturing high performance liquid chromatography
FAP	– familial adenomatous polyposis
HNPCC	– hereditary nonpolyposis colorectal cancer
LEF	– lymphoid enhancer factor
MAP	– *MYH* associated polyposis
MLH1	– mismatch repair gene
MMR	– mismatch repair genes
MSI	– microsatellite instability
MSH2	– mismatch repair gene
MTH1	– enzyme of BER system
MYH	– MutY homolog *Escherichia coli* (gene)
OGG1	– enzyme of BER system
PCR	– polymerase chain reaction
PTT	– protein truncation test
TCF	– T cell factors

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