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Familial adenomatous polyposis of the colon

Rodzinna polipowatość gruczolakowata

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Słowa kluczowe
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Summary

Familial adenomatous polyposis (FAP) is a well-known predisposition to the occurrence of a large number of polyps in the colon and rectum inherited in an autosomal dominant manner. The first symptoms of FAP are diarrhea and blood in the stool. Weight loss and weaknesses occur after the development of advanced tumour. The incidence of FAP disorder is one per 10 000 newborns. There is a high heterogeneity with regard to the number and timing of occurrence of polyps. The classical form of FAP is characterized by the occurrence of more than 100 polyps, which appear in the second decade of life. The average time of occurrence of polyps is 15 years. The earliest symptoms of polyposis were observed in a three-year-old child. The polyps are characterized by large potential for the development towards malignant tumour. Turning into malignancy can occur from the late childhood to the 70s. Attenuated adenomatous polyposis coli cases are characterized by a benign course of disease as opposed to the classical FAP. The occurrence of FAP is associated with mutations in the *APC* tumour suppressor gene, which was described in 1991. The *APC* gene is a tumour suppressor gene located on chromosome 5q21 involved in cell proliferation control. A recessive form of FAP is caused by mutations in the *MUTYH* gene occurring in both alleles of the gene. The *MUTYH* gene is involved in repairing the oxidative DNA damage. *MUTYH* associated polyposis (MAP) is a predisposition to the occurrence of polyps of the colon but the number of polyps is lower in comparison to classical FAP.

The high risk of cancer observed in those syndromes makes them important medical issues. Molecular studies of polyposis of the colon have been performed in Poland for over fifteen years. In 1997 at the Institute of Human Genetics in Poznań, the DNA Bank for Polish FAP patients was established. In the Bank the DNA samples from over six hundred FAP families have been collected so far.

Streszczenie

Rodzinna polipowatość jelita (FAP) jest dobrze poznanym zespołem predyspozycji do występowania choroby nowotworowej rozwijającej się z licznych polipów okrężnicy i odbytnicy, dziedziczonym w sposób autosomalno-dominujący. Pierwszymi objawami FAP są biegunka i krew w stolcu. Wraz z rozwojem choroby nowotworowej dochodzi do utraty masy ciała i osłabienia organizmu. FAP występuje z częstością 1 na 10 000 urodzeń. Czas wystąpienia polipów i ich liczba są w przypadku tej choroby zróżnicowane. Klasyczna forma FAP charakteryzuje się występowaniem więcej niż 100 polipów, które pojawiają się w drugiej dekadzie życia. Średni wiek występowania polipów to 15 lat. Najmłodszy chory miał 3 lata. Polipy charakteryzują się dużym potencjałem rozwoju w kierunku nowotworu złośliwego, który może wystąpić od późnego dzieciństwa do 70. roku życia. Łagodna forma polipowatości rodzinnej charakteryzuje się łagodniejszym przebiegiem niż klasyczny FAP. Występowanie FAP jest związane z mutacjami w genie supresorowym nowotworów *APC*, który został opisany w 1991 roku. Gen *APC* jest zlokalizowany na chromosomie 5q21 i związany z kontrolą proliferacji komórek. Forma recesywna FAP jest spowodowana

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przez mutacje w genie *MUTYH*. Gen *MUTYH* jest zaangażowany w naprawę oksydacyjnych uszkodzeń DNA. Polipowatość związana z *MUTYH* (MAP) jest skłonnością do występowania polipów jelita, ale liczba polipów jest niższa w klasycznym FAP.

Wysokie ryzyko wystąpienia choroby nowotworowej w tych chorobach sprawia, że należą do ważnych zagadnień medycznych. W Polsce badania molekularne wykonywane są od ponad piętnastu lat. W Instytucie Genetyki Człowieka PAN w Poznaniu utworzono Bank DNA dla polskich pacjentów z polipowatościami. W Banku DNA zgromadzono próbki DNA od siedmiuset rodzin z FAP.

INTRODUCTION

Familial adenomatous polyposis (FAP) accounts for about 1% of all colon tumours (1). The frequency of the incidence of this disease 1 in 8000 to 1 in 10 000 births (2). The age of manifestation of symptoms in patients varies considerably and it even varies between siblings. However, it can be assumed that the occurrence of the colon tumour at a young age should be a signal to perform family anamnesis, which allows to identify whether this is a hereditary predisposition (3). The occurrence of a single case of the disease does not exclude a high hereditary predisposition since a given patient may be the first to carry the mutation. FAP symptoms occur earlier than those of HNPCC (Hereditary Non-polyposis Colorectal Cancer) and appear in the second decade of life. However, cases have been observed of the occurrence of the disease as early as five years of age, and in our group there was one three-year old patient to have polyps diagnosed (4). The genetic basis of the occurrence of adenomatous polyps is the presence of mutations in the *APC* genes in cases of FAP patients and in the *MUTYH* gene in the cases of the recessive form of colon polyposis.

The suppressor tumour genes are involved in the control of cell proliferation. Protein products of suppressor genes take part in the control of the cell cycle as its inhibitors and are also components of system for contact inhibition of cell-growth. The suppressor genes perform the function in maintaining the number of cells at a constant level. Disturbances in these mechanisms lead to an increase of the frequency of cell divisions as well as to an increase of the number of errors during division. This leads to accumulation of alterations in the genetic material and selection of an immortal clone of very frequent cell divisions, which is capable of residing in other tissues.

In the case of suppressor genes the phenotype of mutation is masked by an appropriate allele of the gene. In the initiation of the tumour o process/tumourigenesis two independent mutations occur within the locus of the suppressor gene (2). In the case of the carrier state of the mutated allele of the *APC* gene the risk of occurrence of the second mutation, and thus of initiation of tumour disease is very high.

HISTORY OF INVESTIGATIONS OF THE *APC* GENE

FAP was recognized as a heritable pathogenic syndrome already in the 1920s. In 1972 Gardner syndrome was described, which is a form of FAP characterized not only by the presence of hundreds or even thousands of polyps in the intestine, but also of osteomas and retinal hypertrophy (Congenital Hypertrophy of the Retinal Pigment Epithelium – CHRPE). The occurrence of FAP was associated with the q21-q22 region of chromosome 5 on the basis of a large deletion discovered during cytogenetic analysis as well as research results from the linkages of RFLP markers in a patient with Gardner syndrome and with an advanced polyp growth in the colon (5). At the end of the 1980s studies of associations revealed a region on the long arm of chromosome 5, which encompassed the *APC* and *MCC* genes, which are distant from one another by 150 kbp. In 1991, the following three genes were studied in FAP patients: *DP1*, *SRP19* and *DP2.5*. These genes were found in the region which underwent deletion. In four unrelated FAP patients four mutations were found in the *DP2.5* (now known as the *APC* gene) leading to the Stop codon from which one was transmitted to offspring (1). In the following year 79 FAP patients were examined and in 67% of them mutations in the *APC* gene were observed. In the study, 92% of the mutations were those which resulted in the truncation of the protein product of the *APC* gene (6). In many countries, investigations were undertaken to research the occurrence of mutations in the *APC* gene and a database was established in which 826 inherited mutations and 650 somatic mutations were collected (7). The *APC* gene protein function has been studied since 1993 when it was observed that it binds to β-catenin, which indicated participation in cell adhesion (8).

In Poland, investigations of the *APC* gene were started in the middle of 90s of the 20th century, and a DNA Bank of patients was established in 1997 at the Institute of Human Genetics of the Polish Academy of Sciences in Poznań (9). In 2011 the Bank was transformed into a DNA Bank of patients with intestinal polyposis (10). Currently, in the bank there are samples from over 758 families of which 677 are families with FAP, 28 with JPS, 48 with PJS and 5 cases of Cowden syndrome. Molecular studies have allowed to identify mutations in the *APC* gene in 323 families. 194 types of mutations have been identified of which 147 are deletions, 32 insertions and 115 substitutions. Also methodologies of studies of hereditary predispositions to the occurrence of intestine polyposis have been developed (5, 11-18).

APC STRUCTURE AND APC PROTEIN FUNCTION

The *APC* gene (adenomatous polyposis coli) is located on chromosome 5 in the q21 region and contains 21 exons (19). The characteristic attribute of the *APC* gene is the occurrence of a large exon 15, which encompasses over 70% of the coding sequence. The expression of the gene is observed in all tissues. The product of transcription is mRNA of the length of 8538 nucleotides (1, 20). The first exons of the gene may form tissue-specific alternative transcripts (21), e.g. in brain a product of the *APC* gene occurs for which Start codon is located in exon BS (brain specific). Elimination of codon 1, which encodes the super helix domain, known to serve either homo- or heterodimerization affects the functionality of the protein product (19). Alternative splicing of primary exons of the the gene may be related to the occurrence of the attenuated adenomatous polyposis coli (AAPC) (22). A similar effect was observed in one of the two families with mutations at the 3' end of exon 9 where, in one case, a modifying effect was shown of alternative splicing leading to a milder FAP phenotype. In this connection it is suggested that the kind, location of mutation as well as the effect of alternative splicing exert an influence on the course of the disease.

Very interesting is the observation of differential splicing as a result of which exon 14 and exon 15, which encompasses almost 70% of the *APC* gene are excised, and the fragment that remains binds with the 3' end of the *SRP1* gene. Excision of exon 14 or 15 leads to the development of two isoforms differing from each other by their ability of binding microtubules and β-catenin as well as by the sequence of 3' region which does not undergo translation and which can exert an influence on the stability of mRNA and the function of the product (23). In this case alternative splicing of the gene is associated with the regulation of the APC protein activity and suggests that it fulfils many different functions in the cell, especially, that in the alternative splicing of the protein over 75% of exons take part.

The full-length APC protein contains 2843 amino acids and can be found in the cytoplasm and in cell nucleus (1, 20). So far several proteins have been identified to interact with the APC protein. These are DLG protein, microtubule protein, GSKβ-3, β-catenin, γ-catenin, p34, Tid56, Auxin protein. Interactions with many proteins indicate that the APC protein participates in the regulation of many cell processes including: cell division, migration, adhesion and cell fate determination (24). In the APC protein several functional domains have been identified. The base domain encompasses amino acids 2200-2400 (24). The 5' end of the protein, between amino acids 1-171, is involved in oligomerization. In the APC protein there are two β-catenin binding sites – in the fragment comprising three 15-nucleotide repeats between amino acids 1020-1169 and in the region of 20 amino acid repeats between amino acids 1324-2075. Binding with microtubules, which occurs with increased gene expression, takes place by means of the fragment encompassing amino acids 2130-2483. Amino acids 2560-2843 are the site of binding with the EB1 protein, while amino acids 2771-2843 bind with the DLG protein (1). The region associated with the process of apoptosis has not been distinguished, although it was observed that expression of the appropriate APC protein in the intestinal neoplastic cell line leads to the occurrence of this phenomenon. A product of the *APC* gene of 300 kDa mass participates in the inhibition of cell growth in the mucous cells of the colon.

Both proteins bind with a cell adhesion protein E-cadherin. Fearon proposed a model in which the APC protein participates in signal transduction and through degradation of β-catenin affects the activity of T-cell transcription factor 4 (25). The protein that regulates the formation of the APC protein and β-catenin complex is protein kinase ZW3/GSK3β. Phosphorylation of the APC protein activates β-catenin binding. The activity of the GSK3β kinase is regulated by the DSH protein, which interacts with the protein product of the *WNT1* gene. The APC protein is bound with ZW3/GSK3β kinase and is capable of inhibiting the transcription induced by β-catenin. In case of the loss of the function of the *APC* product the transcription factor Tcf4 (*TCF7L2*) is activated. The cell is stimulated to proliferate as a result of activation of the *c-MYC* gene transcription by Tcf4 (26). The product of the *c-MYC* gene resides in the cell nucleus and is capable of binding with DNA, activating the growth gene – ornithine decarboxylase (*ODC1*) and the *CDC25A* gene. It is also an inhibitor of the *GAS1* gene. It was also shown that the activated β-catenin-Tcf4 complex induces Tcf1 expression (27). In the mucous cells of the colon the *APC* is a negative regulator of the cell cycle through the regulation of β-catenin level, which is activated by the proliferation signal derived from the transmembrane protein E-cadherin. In case of the loss of functionality of this gene the balance between cell division and cell apoptosis becomes disturbed.

MUTATIONS OF THE *APC* GENE

Since 1991 when the association between mutation of the *APC* gene and FAP was discovered, the development of molecular studies has been observed. Over the last 17 years the development of techniques could be observed, which resulted in an increased number of publications on the spectrum of mutations in a growing number of countries and ethnic groups. Investigations performed over several years using such techniques as single-stranded conformation polymorphism (SSCP) analysis, heteroduplex analysis (HD) or the denaturing gradient gel electrophoresis (DGGE) and DNA sequencing allowed to establish mutation databases. At the present time, the best *APC* mutation database is at the Institute of Genetic Studies in Cardiff (http://www.hgmd.cf.ac.uk/ac/index.php). This is the most up to date mutation database for most mutations described (28). In the database for the *APC* gene infor-

mation exists for 858 types of mutations. The highest proportion of mutations of the *APC* gene are small deletions. A total of 356 such mutations have been described and the majority of them lead to a change in the reading frame and the creation of a premature termination codon. Large deletions (16) are much rarer as are splice site mutations (9), small deletions combined with insertions (29), large insertions (11), complex rearrangements (5) and 3 mutations in regulatory sequences, which were observed. The next type as far as the frequency of occurrence is concerned are substitutions of which 235 have been described. They lead to changes of amino acids or to the development of the Stop codon in the mutation site. The total of 131 small insertions has been described so far. In the case of substitutions, which constitute 30% of mutations, the Stop codon arises at the mutation site, in the case of deletions or insertions, which constitute 68% of mutations, are formed in its vicinity as a result of the change of the reading frame. There is a region in the gene which is characterized by an increased frequency of mutations called the MCR (mutation cluster region), which encompasses codons 1055-1309. In this region there are 23% of all germline mutations. In addition, among the germline mutations an increased frequency of two mutations has been observed. These are 5bp deletions at codon 1309 (10%), 5bp and deletions at codon 1061 (30). The majority of mutations can be found in the 5' region of exon 15 of the *APC* gene. A characteristic attribute of FAP is the loss of heterozygosity (LOH) in the *APC* gene developing as a result of somatic mutation in the second allele of the *APC* gene. The distribution of frequency of these mutations differs from germline mutations. Up to 60% of somatic mutations are found in the fragment which encompasses 8% of the gene between codons 1286 and 1513. Somatic mutations occur in two hot spots in codon 1309 and codon 1450. Mutations in the *APC* also occur in cases of colon tumours unrelated to polyposis. In cases of the neoplasms, individual tumours occur since there must be a mutation of one of *APC* alleles followed by a loss of heterozygosity as a result of somatic mutations. In case of the Lynch syndrome (HNPCC), the process is accelerated by inherited mutations in the DNA repair genes (31). According to the latest investigations, it is not necessary for LOH to occur in *APC* for the initiation of the colon tumour unrelated to FAP to take place. In 50% of rare cancers in which changes in *APC* have been observed, the onset of cancer is connected with the heterozygous mutations in the β-catenin gene (CTNNB1). Mutations occur in the site of phosphorylation of β-catenin by GSK3β kinase. They cause the switching off of the regulatory function of the *APC* gene, which leads to accumulation of β-catenin, thus to the expression of the MYC gene and the development of the colon tumour (32). β-catenin is found downstream the *APC* gene in the pathway of growth contact inhibition and in the case of mutation in this gene the tumour process occurs independent of the condition of the *APC* gene.

An attempt was made at describing the relationship between the mutations terminating translation and the clinical picture of the disease and manifestation of non-extracolonic symptoms. It was observed that the occurrence of Stop codon before 157 codon causes a decrease in the number of polyps and a milder course of the disease. The typical course of polyposis with numerous polyps takes place when the Stop signal occurs between codons 169 and 1600. Mutations which occur between codons 1403 and 1587 lead to the intensification of extra-colonic symptoms. The occurrence of desmoid tumours is associated with mutations in the region between codons 1445 and 1578. Mutations at the 3' end of the *APC* gene give a varied picture of the course of the disease both with respect to the number of polyps as well as to the manifestation of extra-colonic symptoms.

PROGRESSION OF MORPHOLOGICAL AND GENETIC CHANGES IN FAMILIAL ADENOMATOUS POLYPOSIS

The inheritance of the mutated allele of the *APC* gene does not result in the clinical picture of the disease. The appearance of clinical symptoms is associated with the appearance of a sequence of further events in the cell. **Both in the case of FAP and HNPCC, cells are characterized by a high risk of occurrence of the loss of heterozygosity in the locus of the *APC* gene. During the development of tumour, changes take place in the suppressor genes, oncogenes and DNA repair genes.** Deletions are observed in the regions of occurrence of genes which inhibit proliferation located on chromosomes 5q (*APC*, *MCC*), 17p (*TP53*) i 18q (*DCC*) and point mutations are observed within the *K-ras* protooncogene. In the case of FAP the risk of heterozygosity is very high since one of the alleles is inherited in the mutated form. The risk of initiation of the tumour is very high also as a result of inheritance of mutations. Despite various causes the first stage of initiation of neoplastic disease is the loss of heterozygosity in the locus of *APC*, which leads to the increase of cell proliferation as a result of activation of protooncogene *c-MYC* transcription. Frequent cell divisions lead to the selection of a clone with a damaged *MCC* gene, which causes dysplasia and at a later stage the formation of first grade adenoma. The increase of the speed of cell division results in the further accumulation of genetic errors and is followed by activation of the protooncogene *K-ras* and *DCC* (deleted in colorectal cancer) deletion. The next stage of tumourigenesis is the loss of function of the product of the *TP53* gene, which leads to the formation of an adenomatous tumour, and further accumulation of errors leads to the formation of an invasive tumour. The difference in the molecular basis of FAP and HNPCC results in the faster development of individual tumours in HNPCC to the invasive form than in the case of FAP.

STRUCTURE AND LOCATION OF THE *MUTYH* GENE

The *MUTYH* gene (*MutY*, *E. coli*, *Homolog*) is a homologue of a bacterial mutY gene involved in the repair system of the oxidative DNA damage. The *MUTYH* gene is frequently referred to as *hMYH* or *MYH* but these names are considered to be inappropriate. In human, the *MUTYH* was first described in 1996. This gene encompasses 7100 bp on chromosome 1 in the region p34.3-p32.1 and consists of 16 exons. None of the 15 introns of the *MYH* gene exceed 200 bp. The gene encodes 535-amino acid protein, which is 41% identical with the mutY protein found in *E. coli* (33). Most frequent mutations are two substitutions, Y165C and G382D observed in 80% of patients with mutations of both gene alleles (3, 34, 35).

CLINICAL DESCRIPTION

FAP is a well recognized hereditary predisposition to the occurrence of tumours of the alimentary tract. In patients hundreds or even thousands of polyps are found in the colon and rectum. Great heterogeneity is observed as far as the number of polyps and the age of patients in whom they are found are concerned. Apart from the symptoms in the colon, in case of FAP various intensity of occurrence of extra-intestinal symptoms is observed. Such a phenotype of the disease is associated with the dominant mutations in the *APC* gene (1, 36, 37). FAP with numerous extra-intestinal symptoms such as cysts of sebaceous glands of skin, osseous polyps and desmoid tumours as well as changes in dentition consisting in the change in the number of teeth and manifestation of long and sharpened roots was defined as Gardner syndrome (MIM 175100.0006) (38). In the most recent review literature the term Gardner syndrome ceases to be used and such a disease cannot be found in the MIM base. In FAP a typical and attenuated form can be distinguished. Apart from that in the typical form a severe course of the disease with very numerous extra-colonic symptoms was distinguished (39, 40). Studies of particular groups allowed to select from a group of patients the persons in whom no mutations in the *APC* gene were discovered. After their analysis, it was found that in some cases a recessive inheritance of the disease is observed. This led to a conclusion that mutations in another gene can lead to a similar phenotype as mutations of the *APC* gene. This gene appeared to be *MUTYH* (*MutY*, *E. coli*, *homolog*) (24, 34, 35, 41, 42). Thus a new disease was distinguished – a recessive, autosomal type of adenomatous form of polyposis of the colon (colorectal adenomatous polyposis, autosomal recessive).

PHENOTYPE MUTATION IN THE *APC* GENE

Familial adenomatous polyposis (FAP) (MIM 175100) symptoms in the form of numerous (hundreds or thousands) of polyps in the mucosa of the colon are manifested at the end of the second decade of life. The average age of patients in whom polyps occur is 15 years (43). Polyps are characterized by a high potential to the development of a malignant tumour. Practically, the risk of occurrence of a tumour in FAP patients amounts to 100%. The malignant tumour may occur from the late childhood till the age of 70. The earliest occurrence of polyposis symptoms was observed in a 5-year old patient (44). The first symptoms of the occurrence of polyps are diarrhea and blood in the stool followed by loss of weight and general weakness. The incidence of the FAP is 1/10 000 births (43). The typical form of FAP is characterized by the manifestation of over 100 polyps, which appear most often in the second decade of life as adenomatous polyps and can co-occur with lymphoid ones (45). Most frequently tubular polyps of the diameter of even several centimetres occur. Also tubular-villous and villous polyps are observed (46). Colon polyps may also be found as flat tubular polyps (47). In the typical form of polyposis a phenotype of severe FAP was found, where the number of polyps exceeds a thousand (39). A severe course of FAP is characterized by the earlier age of patients in whom the tumour in the colon occurs and the average age of occurrence is 34 and there is greater frequency of extra-intestinal symptoms.

In FAP patients extra-colonic features vary considerably and these are:

1. Retinal pigment discolouration of the eye fundus (congenital hypertrophy of the retinal pigment epithelium – CHRPE) occurs in more than half of the carriers of the mutation in the *APC* gene (48). Retinal pigment discolouration does not appear if the product of the mutated gene is smaller than 50 kDa, and exon 9 is considered as the boundary between mutations not causing or causing discolouration. In the 3' region of the *APC* gene, the boundary of the occurrence of hyperpigmentation is codon 1387 (48). The length of the product of the APC gene affects the discolouration picture. Gene protein products of a length less than 1014 amino acids present a picture of a small round pigmented spot or large round pigmented spot. Protein products which encompass more than 1014 amino acids are associated with an increased incidence of the remaining two types of retinal changes in the eye fundus, i.e. oval pigmented with a surrounding halo and a large round de-pigmented spot (49). Codon 1014 is the binding site of two cytoplasmic proteins: a and b-catenin, which are important for protein activity in the adhesion of E-cadherin cells. Products longer than 1014 amino acids can bind with these proteins which may affect the change of phenotype discolouration.
2. Changes in dentition involved the appearance of supernumerary teeth most frequently, and of osteomas and changes in the structure of the teeth.
3. Polyps in the upper part of the gastrointestinal tract. Duodenal and gastric polyps can occur

in the form of the stomach fundus polyps and adenomas (14, 15). Stomach polyps occur in approximately 50% of FAP patients and do not have high potential to form neoplasms. Adenomas are observed in 6% of cases and form neoplasms even more rarely than polyps of the stomach fundus (50). Duodenal polyps are observed in 33% to 44% FAP patients (29, 51-53). However, a group of probands was described where the frequency of duodenal polyps was as high as 80% (54). Most frequently polyps are situated in the descending part of the duodenum. In some patients also larger polyps are observed in the vicinity of the larger papilla of the duodenum.

4. Desmoid tumours are observed in about 10% of FAP patients, and their appearance usually follows a surgical operation (55). In FAP patients an increase in the frequency of occurrence of desmoid tumours is observed in comparison to general population. Moreover, gender is also found to modify the frequency of occurrence. The disease occurs less frequently in men with FAP than in women and the ratio is 1:3 (55, 56).
5. Thyroid gland tumours are observed in 94% of women (7, 57, 58).
6. Tumours of the central nervous system are rare and their frequency is about 1%. And these tumours are gliomas. The occurrence of these tumours with FAP symptoms in the colon was described as the second form of the brain tumour colon polyposis syndrome known as Turcot syndrome 2 or brain tumour-polyposis syndrome 2 (BTPS2) (59-61). The occurrence of brain tumour in FAP cases is associated more with the occurrence of the locus modifying the course of the disease than with a specific spectrum of mutations in the *APC* gene. The more so that in rare cases of gliomas or medulloblastomas of the brain no mutations in the *APC* gene have been observed (61, 62).
7. Hepatoblastomas are rare neoplasms occurring in children. An increased risk of the occurrence of these cancers in the APC mutation carriers was observed, but the incidence is low and does not exceed 1% (39, 63-65).

AN ATTENUATED FORM OF THE FAMILIAL ADENOMATOUS POLYPOSIS COLI (AAPC)

An attenuated form of the familial adenomatous polyposis coli (AAPC) is characterized by the occurrence of a small number of polyps ranging from several to one hundred. From among the extra-intestinal symptoms, polyps of the stomach fundus are rare (49). This form of familial polyposis of the colon is connected with the occurrence of mutation at the 5'end of the *APC* gene. It is assumed that codon 159 is the boundary between AAPC and FAP causing mutations, although it is difficult to establish this boundary unequivocally because this form of the disease occurred also in cases when the mutation led to the formation of the Stop codon in exon 9 of the *APC* gene (66).

PHENOTYPE OF THE *MUTYH* GENE MUTATION

Recessive polyposis (MIM 608456) MAP (MUTYH associated polyposis) is an autosomal recessive predisposition for the occurrence of numerous polyps in the colon the numbers of which are smaller than in the case of FAP and do not exceed 100 (42). Cancer risk in the carriers of mutations of both alleles of the *MUTYH* gene is 93 times higher than it is in the general population. The intestinal neoplasm occurs almost always before the 60th year of life (51). It was also observed that mutations of both alleles of the *MUTYH* gene increase the risk of endometrial tumour, which at a small number of polyps or their absence, may make the phenotype resemble the Hereditary Non-polyposis Colorectal Cancer (HNPCC) (67). Differences in the occurrence of the pathological symptoms are also observed in the relatives of carriers of the same mutations (68).

FAP MOLECULAR DIAGNOSTICS

The material for studies is DNA isolated from peripheral blood cells. In the search for mutations are employed screening methods such as: heteroduplex (HD) analysis, single-stranded confirmation polymorphism (SSCP) of DNA and High Resolution Melting (HRM) (fig. 1). The HRM analysis deserves a longer comment. The principle of the operation of this method is based on monitoring the behaviour of the previously amplified DNA fragments in the denaturation process. Analysis is possible due to the presence of a fluorescent dye in the reaction mixture, which intercalates only the double-stranded DNA, giving a strong fluorescent signal. During the DNA melting the fluorescence level falls depending on the amount of hydrogen bonds between bases. A very accurate measurement of fluorescence in particular stages of changes in temperature by 0.01°C allows to observe differences in the melting behaviour of samples. When comparing melting profiles of particular fragments with one another, it is possible to select those that show a difference in the course of denaturation, which thus reflects changes in the sequence. HRM is a screening technique and does not allow obtaining precise information about changes in the sequence that is why all the untypical melting profiles require confirmation by means of sequencing. However, high sensitivity of the method, which in some studies reaches 100%, allows reducing considerably the number of DNA sequencing, and thus to lower the cost of analyses and, at the same time, to maintain (and even increase) the efficiency of mutations detection when compared to older screening methods (3, 24). An important issue is the study of large rearrangements in the *APC* gene. Searching for large changes within particular exons or even entire genes was much facilitated when in 2002, Schouten described the method of simple quantitative analysis of as many as 40 DNA frag-

ments amplified by MLPA (Multiplex Ligation-dependent Probe Amplification) primers. Since then there has been a rapid development of this technique and at the present time the method is used in studies of predisposition to the occurrence of hereditary diseases, characteristic of cancer, the RNA analysis, methylation analysis and pharmacogenetics. The method is based on hybrydization of probe halves to selected fragments of genomic DNA, their ligation and then amplification. This makes the quantitative evaluation of PCR products possible. Hybrydization of the probes to a specific DNA fragment opens up possibilities for the study of known mutations that occur with increased frequency in cases of certain diseases. The MLPA kit P043 for the *APC* gene containning probes for testing particular exons of gene and two probes for the detection of mutations c.3183-3187delACAAA and c.3927-3931delAAAGA. These two mutations in some populations constitute even up to several per cent of all detected mutations in FAP patients. In our group of FAP patients, these two mutations are present in almost 15% of them. Familial adenomatous polyposis of the colon has been investigated in Poland for over 10 years at the Institute of Human Genetics of Polish Academy of Sciences in Poznań. The DNA Bank of Polish patients with familial polyposis contains material from over 600 families. Around 50% of FAP families without an identified mutation in the APC gene pose a diagnostic problem. Recent reports indicate that scientists should be more interested in changes beyond the coding sequence because their impact on disorders in the appropriate expression may be of great importance.

FAP PROPHYLAXIS

Determination of mutation carriers is connected with application of appropriate prophylaxis for them. At the present time patients at risk of disease are screened by means of sigmoidoscopy, which is performed every 12 months beginning from puberty. This allows to discover symptoms long before the development of an invasive neoplasm. Once polyps appear, the only effective way to prevent the occurrence of the invasive cancer is to remove surgically the entire colon. Prophylactic colectomy performed at an appropriate time prolongs the life expectancy of FAP patients, on average, from 45 to 60 years.

During surgery, the entire colon is removed and a J pouch is formed using the small intestine. The mucous membrane of the small intestine has considerable plasticity and it becomes morphologically similar to the mucous membrane of the colon. Surgery is currently the only way to improve the life expectancy of patients, and although effective, it causes severe mutilation, especially when performed at an advanced stage of the disease when the anus must also be removed. In view of this, there is much interest in studies on the impact of composition of food and of non-steroid anti-inflammatory drugs which have been shown to reduce the numbers of adenomatous polyps. When studying the starch content in food and incidence of colon tumours, it was found that there is an inverse correlation between the amount of starch in the diet and the frequency of occurrence of colon cancer. Studies are conducted on mouse models. A number of mouse models have been developed, including a functional/knock out gene *Apc*Δ716 (69). These mice are heterozygotes for the Apc mutation in codon 716 which results in a typical course of the disease in terms of polyp appearance occurring at about the third week of life in the mouse. Homozygotes of mutations are embryologically lethal. Diets containing high levels of starch and low levels of fat did not exert an influence on

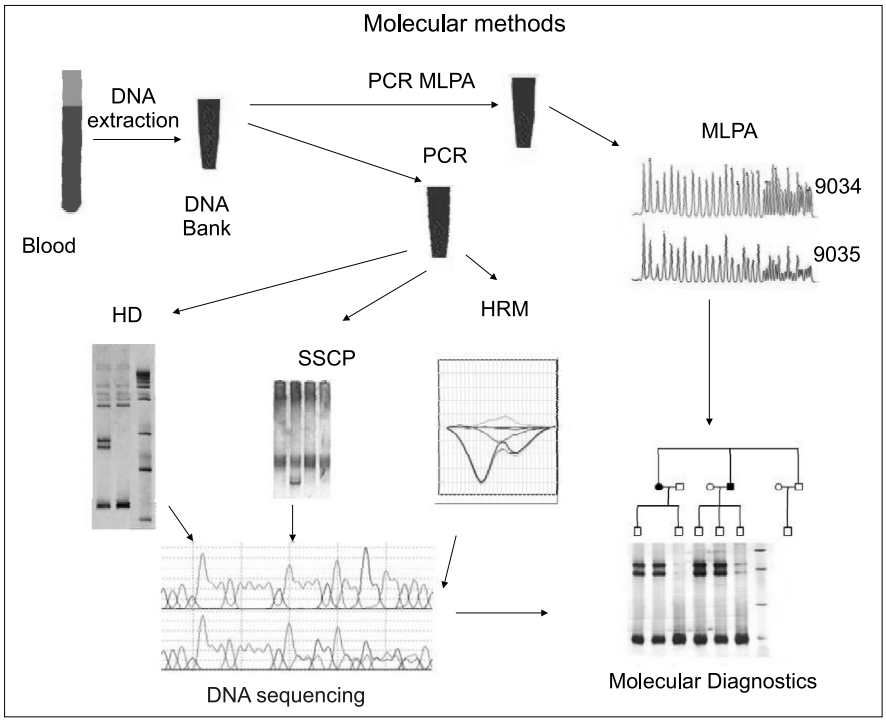

Fig. 1. Molecular diagnostics of FAP.

the frequency of disease but it reduced the numbers of polyps by 64% as well as their size in sick individuals. The number of polyps is also decreased as a result of administration of non-steroid anti-inflammatory drugs. Application of Aspirin, Piroxicam and Sulindac reduced the number of polyps in the colon of experimental mice with mutated *Apc* from 40 to 60% (70).

Attempts are also made at using gene therapy for FAP treatment. The fact that the disease is preconditioned by a mutation in one gene makes attempts at repairing the error significantly easier. To the line of mucous cells of the colon, SW480 with the mutated APC, a complete *APC* functional gene was introduced using liposomes as a vector. The efficiency of this system is low and the gene is not integrated with the genome of the target cell, however, liposomes are much safer than the retroviral vectors. The *APC* gene transfer allowed to obtain an expression of the correct *APC* gene in the SW480 cells after 72 hours, and it was observed for a period of one week at a level which guaranteed a biological effect.

Neoplastic diseases of the colon are well recognized but it is still necessary to understand all factors which influence the initiation and development of the tumours and interactions between these factors. Studies of mutations occurring in the course of the development of the neoplastic disease will make it possible to select the most effective prophylactic method or minimization of pathological consequences of the disease. Most hopes are in the application of the gene therapy. In this case, in order to eliminate totally the cause of the disease a correction of genes in most cells of the organism should be performed in order to avoid numerous extra-intestinal symptoms. Despite recognition of mutations causing the disease, for the complex correction of the known error a breakthrough must be made in the technology of gene transfer.

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