Do we know all there is to know about Familial Adenomatous Polyposis?

SUMMARY

Familial Adenomatous Polyposis (FAP) and Attenuated FAP (AFAP) are caused by a germline mutation in the Adenomatous polyposis coli (APC) gene. Recently, a new pathway characterized by a biallelic mutation in the MYH gene, with a recessive model of inheritance was discovered for this inherited syndrome. This report describes a Tunisian patient with an attenuated FAP phenotype, presenting seven colon polyps and an adenocarcinoma but no detectable germline mutations in the FAP target genes. A well known somatic mutation was found in the APC mutation cluster region (MCR). This case shows that further studies are needed to fully understand all the pathways of the FAP syndrome.

RÉSUMÉ

La polypose adénomateuse familiale (PAF) et celle atténuée (PAFA) sont provoquées par une mutation germinale au niveau du gène Adenomatous polyposis coli (APC). Récemment, une nouvelle voie a été découverte, il s'agit de la voie PAM (polypose associée à MYH) à transmission récessive, provoquée par une mutation au niveau du gène MYH. Cette étude s'intéresse à un malade tunisien présentant un phénotype de PAF atténuée sans aucune mutation au niveau des gènes majeurs prédisposant à ce syndrome. L'étude somatique du gène APC au niveau de la région MCR a révélé la présence d'une variation. Ce cas clinique montre que d'autre études sont nécessaires, pour mieux comprendre les différents voies et formes de PAF.

Introduction

Familial Adenomatous Polyposis (FAP) is considered an inherited colorectal cancer syndrome [1]. It is an autosomal dominant syndrome characterized by hundreds of colorectal adenomatous polyps that progress to colorectal cancer (CRC) if left untreated [2]. FAP accounts for 1% of all CRC and affects about 1/10000 individuals [2].

The colorectal polyps may be accompanied by extracolonic manifestations, such as upper gastrointestinal adenomas and tumors of the small intestine and stomach [3], desmoid tumors [4], congenital hypertrophy of retinal pigment epithelium (CHRPE) [5] and also bone lesions and teeth abnormalities [6].

APC (adenomatous polyposis coli), the gene responsible for FAP, is located on chromosome 5q21 [7] and encodes a large protein that is part of the Wnt signalling pathway (the wingless signal transduction pathway) [8], through the regulation and interaction with other proteins such as beta-catenin that belong to this pathway. Germline mutations in APC are found in most FAP patients [9]. Over 900 germline mutations have been cited so far [10]. Most (95%) of them are nonsense or frame-shift mutations that result in a truncated protein product with an abnormal function.

Besides the classical FAP phenotype, an attenuated form of FAP (AFAP) has also been found [11], characterized by the presence of less than 100 adenomatous polyps in the colorectal system but with a significantly increased risk of developing CRC. AFAP patients harbour germline mutations in the 5’ and 3’ ends, as well as exon 9 of the APC gene [12].

Recently, biallelic mutations in the base excision repair gene MYH (called also MutYH) have been shown to result in a predisposition to multiple adenoma and CRC [13, 14]. MYH-associated polyposis (MAP) accounts for 7-17% of APC negative FAP families [13, 15] and approximately 4% of families with the AFAP phenotype [13]. Moreover, the age of onset is older in MAP than in pathogenic APC mutation carriers [13].

The authors have collaborated on the same level.
In addition, 30% of FAP patients have no family history, suggesting the presence of de novo genetic events. These data show the variability of clinical and genetic manifestations of FAP, making genetic counselling for this disease difficult. Many questions remain about predictive genetic testing for these patients and their family. This study includes a case report of a patient with an AFAP phenotype but no familial history who underwent molecular analyses of APC, MYH and β-catenin.

Materials and Methods

The following gene analysis was performed with the patient’s consent.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA blood mini kit (QIAGEN; Valencia, CA, USA) according to the manufacturer’s instructions. Tumor DNA from paraffin-embedded tissue sections and the corresponding adjacent normal colon tissue DNA were extracted with the DNeasy® Tissue (QIAGEN; Valencia, CA, USA) according to the manufacturer’s instructions. A NanoDrop (ND-1000) spectrophotometer (Wilmington, DE, USA) was used to quantify the DNA.

Multiplex ligation-dependent probe amplification (MLPA)

The SALSA MLPA P043 APC probemix kit (MRQ-Holland, Netherlands) was used according to the manufacturer’s instructions to screen large deletions/duplications of one or more exons of the APC gene. The MLPA method is based on sequence-specific hybridization to genomic DNA. Fragment analysis was carried out on the ABI 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA), using GeneMapper software, version 4.0 (Applied Biosystems, Foster City, CA, USA). Detection of probe recognized sequences was apparent by a 35-50% reduction in the relative peak area of the corresponding amplification product.

Screening for germline mutation in APC, MYH and exon3 of δ-catenin genes

The APC and MYH genes were amplified by polymerase chain reaction (PCR) using DNA purified from blood cells and a set of primers that cover the coding region including all intron/exon junctions to detect any mutations in the splicing sites.

For the β-catenin gene (CTNNB1), a fragment of 200bp of exon 3 was amplified. This region of the β-catenin gene is considered to be a hot mutational spot and encompasses the region for GSK-3β phosphorylation which is important for the Wnt pathway.

Sequencing reactions were carried out in forward and reverse orientations using the BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster city, CA, USA). The products of the sequencing reactions were cleaned up using the sephadex™ G-50 (GE healthcare, Life Sciences) in a MultiScreen™-HV 96-well filter plate (MILLIPORE, Billerica, MA, USA), then run up on the ABI 3730 DNA sequencer. The resulting sequence data were analyzed with the SeqScape software, version 2.5 (Applied Biosystems, Foster City, CA, USA) in comparison to the reference sequences of human APC, MYH and β-catenin gene (Accession Numbers respectively: M74088, NM_012222 and NM_001012329) downloaded from the NCBI Genbank website (http://www.ncbi.nlm.nih.gov/). Mutations were checked in both sense and reverse directions using PCR products from independent reactions.

Screening for somatic mutation in the mutation cluster region (MCR) of the APC gene

The MCR region located in exon 15 of the APC gene was screened for mutations starting from tumor DNA and using four overlapping fragments based on the same protocol described above. Sequencing was performed on DNA isolated from the patient’s paraffin-embedded colon tumor tissue. The analysis covers codons 1240-1513, including all the codons in the MCR [16].

Results

Clinical Diagnosis

The patient was a 59 year old man who consulted at the Charles Nicolle Hospital of Tunis due to rectal bleeding and abdominal pain. Colonoscopy, showed a sigmoid adenocarcinoma and seven polyps in the transverse and ascending colon. Histological examination showed that the adenocarcinoma was ulcerated and moderately differentiated with no colloid component of the mucosa. The tumor stage was T3N0Mx and B2 Dukes. The seven polyps correspond to low grade dysplasia tubular adenoma. The patient had no extracolorectal symptoms and no familial history of CRC. The histo-clinical diagnosis suggested a PAF syndrome and a total colectomy was proposed to the patient.

Genetic Diagnosis

Large genomic rearrangements in APC were screened for in blood DNA using the MLPA technique. No differences were found between the amplified patterns of the patient and normal control (data not shown). This shows that no large genomic rearrangements were detected in the APC gene of this patient at germline level.

We then sequenced all the coding regions of the APC and MYH genes to detect point mutations or small deletions/insertions. No germline mutations were found in any of these genes. Indeed, no putative germline pathological missense mutations (such as I1307K, E1317Q in the APC gene or Y165C and G382D in the MYH gene) were found.

In addition, we amplified the genomic DNA fragment encompassing exon 3 of β-catenin for germline mutations analysis. No mutation in β-catenin was found.

We also analysed the patient’s tumoral colon tissue DNA to identify somatic mutations. The APC gene MCR analyses showed a deletion of an Adenine at codon 1312 (c.3936delA), resulting in a stop codon downstream (at codon 1320). This mutation was heterozygous (figure 1). To test the hypothesis of mosaicism we used normal colon tissue adjacent to tumor tissue and no c.3936delA mutation was found.

Discussion

In the last decade, the development of genetic screening tests has had a significant impact on the detection of inherited forms of cancer, leading to corresponding prevention and monitoring of...
at-risk patients. However if there are false negative molecular results which misinform and incorrectly reassure the patient and his relatives, it is difficult for physicians to give appropriate genetic counseling. Thus, more genetic tests may be necessary, as shown in the present case report of one patient.

The present case report describes a Tunisian patient with seven tubular adenoma polyps. Although this is usually associated with familial adenomatous polyposis, this patient had no family history of FAP suggesting a de novo manifestation. Furthermore, the limited number of polyps is probably a sign of attenuated FAP. We screened the APC gene in blood DNA by two conventional methods: MLPA for large rearrangements and total gene sequencing. No mutations were detected in this gene. This has been observed in many studies [17, 18] which have reported heterogeneity in the germline mutations associated with a severe phenotype of FAP [17]. In our case, the few number of polyps and the absence of extracolorectal manifestations suggests a particular genetic alteration.

Other genes of the Wnt-signaling pathway have been proposed as possible candidate genes for a predisposition to FAP. In fact a mutation in the β-catenin gene is known to promote tumorigenesis by increasing the expression of oncogenes such as c-myc and cyclin D1 [9]; [20, 21]; [22]. For this reason we analyzed the β-catenin-exon3 region which encompasses GSK-3β phosphorylation and is important for the Wnt pathway. No mutations were found in this exon at the germline level (blood DNA).

We also screened the total MYH gene for germline mutations because the phenotype of MYH-associated polyposis (MAP) closely resembles FAP and attenuated FAP phenotypes. The analysis of this gene did not reveal any germline mutations (blood DNA).

Hence, our patient does not correspond to the classical cases of FAP, AFAP and MAP but to an undefined category of patients (table I). 90% of FAP cases including AFAP are mutated in APC and the remaining cases are mutated in the MYH gene. These genes are considered to be the major genes involved in a susceptibility to FAP.

Although FAP is the most clearly defined and understood inherited colorectal cancer syndrome associated with APC mutations, the existence of symptomatic cases without any mutation in this gene highlights the possible implication of other pathways in FAP (table I) such as the MAP discovered in 2003 by Sieber [13], caused by germline mutation in the MYH gene.

The absence of mutations at germline level in both genes as well as in an important functional region of the β-catenin gene suggests the following hypotheses: first, the possible involvement of other genes, with high/low penetrations, promoting FAP; second, an eventual phenocopy state, corresponding to sporadic colorectal cancer. This hypothesis is supported by the patient being older.

In addition to this germline study, we analyzed the APC mutation cluster region (MCR) sequence for somatic mutations.
Indeed, in previous reports, the APC mutation gene is somatically mutated in over 70% of sporadic colorectal cancers, indicating the role of this gene in promoting tumorogenes. However, these sporadic CRC do not have the FAP or AFAP phenotype.

In our study a known mutation (c.3936delA) was detected in the colon tumor that leads to a truncated APC protein, and that has been already described at the somatic level in sporadic CRC tumors and at the germline level in FAP [10, 23].

How could this somatic APC mutation cause an AFAP phenotype?

This mutation may have occurred in the colon cells or their embryonic precursors and may not involve the germline. In this case, the patient would be a mosaic individual with an initial mutation in some cells limited to colon region. To test this hypothesis other tissues should be analyzed. We compared leukocyte DNA, tumor colon DNA and adjacent normal colon DNA for the presence of the c.3936delA mutation, but this mutation was only detected in the tumor cells. This result as well as the milder phenotype in the present case suggests that the APC mutation described is limited to the polyp region cells and occurred before the somatic mutation that characterizes sporadic CRC.

In diseases such as FAP with elevated rates of de novo mutations, mosaicism must also be distinguished to exclude possible errors when doing genetic counseling [24, 25]. In these cases, a systematic study of DNA from different tissues must be analyzed as well as parental DNA, which was not possible in our patient since his parents were dead.

Our results suggest that a novel attenuated non-familial adenomatous polyposis disease exists since this patient is between classic AFAP (polyps) and sporadic CRC (age, somatic APC mutation, absence of familial presentation).

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