

Letter to Editor

Novel Germline Mutation (Q1260X) in APC Gene Causes Familial Adenomatous Polyposis in a Ukrainian Family

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Abstract

Colorectal Cancer (CRC) in developed countries is the major causes of cancer death. It may have sporadic or hereditary origin. Familial Adenomatous Polyposis (FAP) is a very frequent hereditary syndromes predisposing to Colorectal Cancer and is characterized by the development of numerous precancerous polyps (from hundreds to thousands) in the second decade of life.

Classical FAP is caused by germline mutations in the APC gene (Adenomatous Polyposis Coli) a tumor suppressor gene. Often the result of the genetic mutation is a truncating protein that lacks functional domains, such losing its function and promoting tumorigenesis.

A subject who presents a monoallelic germline variation in APC gene inherits in a dominant manner the predisposition to polyps, which will turn in CRC, through the occurrence of a somatic alteration in the wild-type copy of gene. Molecular analyses of APC gene confirms diagnosis in FAP patients and allow to detect the specific pathological mutation in order to screen the unaffected members of the same family, identify pre-symptomatic high-risk subjects and insert them in endoscopic surveillance protocols. We analyzed the APC mutational status in a Ukrainian woman with a history of CRC and proctocolectomy. By direct sequencing of the encoding exons of APC gene we found a c.3778 C>T heterozygous substitution which causes the substitution of a Glutamine with a stop codon, resulting in a premature truncation of APC protein (Q1260X). This mutation had never been described before and it may be considered as a "novel mutation". We extended the analyses to 1st grade related, proving that one of the patient's son was heterozygous for the same mutation and that to the endoscopic evaluation he showed an early develop of hundreds of polyps. This finding confirmed our speculation about the pathologic effect of Q1260X.

Keywords: FAP; APC; Colorectal cancer; Sequencing analysis; MLPA

Introduction

Colorectal Cancer (CRC) is one of the most common malignancies, without difference among men and women, in developed countries where it is considerate the major cause of cancer death [1].

CRC may have sporadic or hereditary origin and in both cases the severity of the disease can be related to environmental or "life style" factors, such as diet (weight gain, consumption of unhealthy food) or tobacco use [2].

The two most frequent hereditary syndromes predisposing to CRC are Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC or Lynch syndrome) [3].

FAP (OMIM ID: 175100) is characterized by the development of numerous precancerous polyps (from hundreds to thousands) throughout the colon and rectum, earlier in the affected subjects than in the general population, often in the second decade of life [4].

The progression of these polyps to CRC is inevitable in all cases without a surgical treatment and with a late identification [5].

Moreover, patients with FAP have an increased risk for extracolonic malignancies, such as osteomas, polyps in other segments of gastrointestinal apparatus, follicular or papillary thyroid cancer, childhood hepatoblastoma, medullablastoma [6]. FAP is characterized by three different phenotypic appearance [7], known as "classical FAP" (CFAP), "attenuated FAP" (AFAP) and "MUTYH-associated polyposis" (MAP). The most common is CFAP in which more than an hundred polyps may be found through the colon and rectum already in the second decade of life [8,9].

To this variable phenotype also corresponds heterogeneity in the predisposing genes. In fact CFAP and AFAP are caused by germline mutations in the APC gene (Adenomatous Polyposis Coli) a tumor suppressor gene, while the gene involved in MAP onset is MUTYH (Human MutY homologue), a base excision repair (BER) gene [10].

The APC gene is located on long arm of chromosome 5 (5q22.2), consists of 16 exons (open reading frame from exon 2 to exon 16) and encodes a \sim 310 kD protein made of 2843 amino acids [3].

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Received August 28, 2013; Accepted November 11, 2013; Published November 21, 2013

Citation: Russo L, Cifarelli RA, Venere BD, Sgambato A, Susi M, et al. (2013) Novel Germline Mutation (Q1260X) in APC Gene Causes Familial Adenomatous Polyposis in a Ukrainian Family. J Genet Syndr Gene Ther 4: 197. doi:10.4172/2157-7412.1000197

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Another clinical condition associated to FAP is Turcot syndrome, in which the presence of a great number of colonic polyps is related to an increased risk of CRC and medulloblastoma. Most often, this disease is related to APC mutations [11].

In the last decades more than 1000 mutation were found in APC gene of FAP subjects and have been registered in public databases [12,13].

Most alterations (including single nucleotide variations, small insertions or deletions) lead to the production of a truncated protein, through nonsense codon formation, base frame-shift and rarely splicing site alteration [10].

Often the results of the genetic mutation are a protein that lacks functional domains, such losing its onco- suppressor role and promoting tumorigenesis.

As expected from the two-hits Knudson's hypothesis, a subject who presents a monoallelic germline variation in APC gene inherits in a dominant manner the predisposition to polyps which will turn in CRC, through the occurrence of a somatic alteration in the wild-type copy of gene [14].

Normally clinical features and anamnestic notices are enough to place FAP diagnosis. Molecular analyses of APC gene may confirm the clinical suspicion in a symptomatic subject, which may be enrolled as index case for the research of specific pathological mutation through the mutational screening of entire APC coding region.

Once the specific disease-causing mutation in the index case is found, the genetic test will be extended to the unaffected members of the same family in order to identify pre-symptomatic high-risk subjects and to insert them in endoscopic surveillance protocols [15].

Our study is part of a molecular oncologic screening on hereditary CRC forms. Here we report the identification of a novel germline APC mutation in a family with a classical polyposis phenotype that harbours a germline point mutation in the exon 15 of APC gene, generating a premature stop codon and thus a truncated protein.

Case Presentation

A 47 year-old Ukrainian female presented to our genetic counseling department after proctocolectomy surgical treatment. In her medical history there was a diagnosis of synchronous cancers of the colon and rectum with uterine infiltration and multiple lymphonodal metastasis, on rectal-colonic polyposis, uterine leiophibromatosis and suspected pulmonary nodule.

The patient's pedigree (Figure 1) included a 45-year-old brother who underwent to surgical treatment for CRC at the age of 38. The brother's son dead at the age of 14 for medulloblastoma.

The early onset of CRC in our patient and in her brother, together with the presence of a brain tumor event in her family, represented good eligibility criteria for the molecular APC analyses in the index case.

Whole coding region of APC gene in our patient was screened for the detection of alterations in constitutional DNA by direct sequencing.

All APC exons had a wild-type sequence, except exon 15, in which we found two heterozygous sequence variants.

First allelic variant found was the c.5465 T>A heterozygous substitution, which is already reported in mutational APC databases and known as missense mutation (V1822D), but without any relevant clinical significance [16].

Sequence analyses of exon 15 also revealed a c.3778 C>T heterozygous substitution at codon 1260 (Figure 2).

This mutation causes the substitution of a Glutamine with a stop codon (Q1260X), resulting in a premature truncation of the APC protein at amino acid position 1260.

This alteration had never been described before, in fact is not reported in APC mutation public databases neither in any scientific article. Therefore it may be considered as a "novel mutation".

On index case DNA was also performed a specific APC MPLA test [17], to screen the presence of eventual intragenic duplication or deletion, but any alterations were detected.

After the identification of c.3778 C>T substitution as causing disease alteration in our index case, we search it also in 15 and 18-year-old patient's sons. Only the 18-year-old son resulted a heterozygous carrier of the same mother's APC mutation, while the other son resulted homozygous for wild-type allele.

Colonoscopy examination performed in the 18-year-old son revealed the presence of multiple sessile colonic polyps of 2 to 8 mm, some of which were excised and histologically analyzed, with a definitive diagnosis of FAP.



Figure 1: Pedigree of the Ukrainian family with inheritance of FAP. Blackened symbols indicate subjects affected by Colorectal Cancer. Symbols with a black point indicate subjects affected by Familial Adenomatous Polyposis. A black arrow denotes index case. Roman number indicates generations.

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Our patient and her first son entered in an endoscopic follow-up program at the department of endoscopic gastroenterology in our hospital, to periodically check the evolution status and the eventual malignant transformation of identified polyps.

Conclusions

Although among CRC forms has an incidence of less than 1%, FAP is one of better known genetic disease and it is recognized that FAP subjects has a 100% risk of early CRC onset [5]. The introduction in the last decades of systematic endoscopic screening programs showed that CRC morbidity association to FAP can be strongly reduced.

The introduction of molecular tests for APC gene on FAP patients and the extension of the analyses to all first-degree relative, also helped in cancer prevention.

For all these reason it is very important that oncologists, surgeons and genetic counselors collaborate in the management of CRC patients, in order to identify all cases with suspected familiarity for cancer and screen all related for the carrier condition, though asymptomatic.

Through the phosphorylation of β -catenin, wt APC protein allows its ubiquitin-related degradation, such modulating a variety of cell process, as cell cycle control, signal transduction, differentiation, transcriptional activation, cell migration, adhesion and apoptosis [18].

More of 60% of APC mutations were described in exon 15 [19] more frequently in a region spanning from codon 1284 to codon 1580 of the APC protein, corresponding to β -catenin interaction domains [20].

The Q1260X mutation found leads to the production of truncated APC protein of 1260 amino acids (such as most of APC described mutations), lacking of the β -catenin binding and degradation domains, a pathogenic considered feature.

The presence of this truncating mutation is in accord with family history, with clinical presentation of the disease as classical FAP in the mother, with the early endoscopic recognition of polyps in mutation carrier son and with histological results.

Moreover mutational APC databases reports pathological nonsense

mutations strictly near to our site of substitution (C1256X, Y1262X) [21,22].

All these remarks consent to us to speculate that Q1260X mutation has a pathogenic role, predisposing its carriers to FAP and to CRC evolution.

As this alteration was never described it would be of great interest to investigate its occurrence in Ukrainian and in general population.

Another point of interest is that in the analyzed family there was a case of medulloblastoma in a young subject and that he descending by the brother of our index case, who developed CRC at 38 years. During our study was not possible to extend the genetic test to these related, because of the different residence state of the brother and his son premature dead for medulloblastoma.

The recovery of the same germinal mutation in these subjects would allow us to associate the novel alteration also with Turcot Syndrome and thus with a specific extra-colonic manifestation of FAP.

Molecular tests available for APC mutation detection are numerous, but direct sequencing remains the gold standard [10], with a mutation detection rate of 80-90% and because it allows the detection of un-known alterations. For these reasons, we decided to perform the full gene screening with this method.

Moreover on genomic DNA of the index case MLPA test was carried out, in order to assess the presence/absence of intragenic copy number variation in APC gene. In fact about 20% of classical FAP patients are homozygous for wild-type allele of both APC and MUTYH gene, while lack of a intragenic region, such as an entire exon or several exons [23]. The effect of these alterations is an altered production or function of APC protein, but they are not detectable with classical molecular method.

No amplification or deletion in APC fragments were detected, allowing us to exclude the presence of other genetic alteration diverse from truncated mutation described above.

Our finding is important for two reason. The first is the confirmation of the hereditary nature of the index case's disease (with the further clinical management of her FAP family). In fact, although without

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symptoms such as rectal bleeding, anemia, alvo defects or abdominal pain, the 18-year-old son at endoscopic evaluation showed already a great number of polyps and was prematurely involved in a surveillance protocol.

Moreover the discovery of a never described mutation extends the spectrum of APC gene germline alterations knowledge.

In conclusion we described a novel mutation of APC gene confirming the hereditary origin of polyposis phenotype in studied family, enrolling the alteration harbouring family components in strict surveillance protocol to control further growth of polyps and prevent respectively CRC relaps or onset.

Further functional studies on Q1260X APC mutation should carry out to definitively demonstrate its impact on normal APC protein function, for example evaluating the subcellular distribution and accumulation of β -catenin [24].

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This article was originally published in a special issue, Genetic Disorders handled by Editor(s). Dr. Xiaobo Li, Yeshiva University, USA