A De novo Germline APC Mutation (3927del5) in a Patient with Familial Adenomatous Polyposis: Case Report and Literature Review

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Abstract

Introduction: Characterized by the development of hundreds to thousands of colonic adenomas, classic familial adenomatous polyposis (FAP) is one of the most common hereditary syndromes associated with an increased risk of colorectal cancer. Several studies have attempted to correlate specific APC mutations with clinical phenotype.⁶ However, there is considerable variability in the expression of specific phenotypes within families and among individuals with identical mutations.⁷

Case presentation: A 30 year-old Hispanic female presented to the emergency department with a 2-week history of persistent, worsening, left lower quadrant abdominal pain. She had no family history of malignancy. Sigmoidoscopy revealed innumerable polyps in the rectum and sigmoid colon and a large mass in the sigmoid colon. Biopsy of the mass revealed a moderately differentiated adenocarcinoma invading the subserosa. Endoscopy revealed innumerable polyps. Genetic testing of the patient via southern blot revealed a germline APC mutation 3927del5, resulting in a premature truncation of the APC protein at amino acid position 1312.

Conclusion: Genetic information has only recently started being incorporated into clinical care. More research and randomized clinical trials need to be conducted to definitively characterize random mutations. Once these mutations are further understood, FAP patients may be able to be risk stratified and this may ultimately improve the screening, diagnosis, and treatment of this rare condition.

Keywords: familial adenomatous polyposis, FAP, colon cancer, sporadic mutation

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Introduction
Characterized by the development of hundreds to thousands of colonic adenomas, classic familial adenomatous polyposis (FAP) is one of the most common hereditary syndromes associated with an increased risk of colorectal cancer. FAP occurs once in every 10,000–30,000 live births,¹ has a relatively equal worldwide distribution, and occurs equally among males and females. Colon cancer occurs in approximately ninety percent of affected individuals by age 45. These patients are also at an increased risk for extra-colonic malignancies, such as duodenal ampullary cancer, follicular or papillary thyroid cancer, childhood hepatoblastoma, and medullablastoma.

FAP is inherited in an autosomal dominant fashion by germ line mutations in the adenomatous polyposis coli (APC) gene located on chromosome 5q21-22.² More than 800 mutations of the APC gene associated with FAP have been described and the majority of these mutations involve frame shifts and premature stop codons, which results in truncated, non-functional APC gene products.³,⁴ In most FAP patients, the expression of the APC mutation involves an inherited mutation of one APC allele, with a “second hit” deletion of the other allele.⁵ Several studies have attempted to correlate specific APC mutations with clinical phenotype.⁶ However, there is considerable variability in the expression of specific phenotypes within families and among individuals with identical mutations.⁷

We report a case of newly diagnosed FAP in a patient without any family history of the syndrome or its associated malignancies.

Case Presentation
A 30 year-old Hispanic female presented to the emergency department with a 2-week history of persistent, worsening, left lower quadrant abdominal pain with increasing constipation. Her past medical history was significant for iron deficiency anemia and migraines. She was a non-smoker and drank alcohol socially. She had two brothers and had no children. There were no 1st, 2nd, or 3rd degree relatives with a history of colon cancer or any other malignancies. The patient’s family had frequent follow-up medical care with adherence to current cancer screening guideline recommendations. On admission, the patient was afebrile with stable vital signs. Physical exam revealed a soft, moderately distended abdomen with hypoactive bowel sounds and tenderness to palpation over the left lower quadrant. Pertinent laboratory findings included white blood cell count 12.8 × 10³/µL, hemoglobin 9.2 g/dL, hematocrit 29.5%, mean corpuscular volume 67.7 fl, red blood cell distribution width 17.9, and carcinoembryonic antigen 1.25 ng/mL. Computed tomography of the abdomen and pelvis with oral and intravenous contrast revealed innumerable polypoid filling defects within the transverse and descending colon (Fig. 1). Sigmoidoscopy revealed innumerable polyps in the rectum and sigmoid colon with a large mass (5.0 cm by 2.0 cm) in the sigmoid colon (Figs. 2 and 3). Biopsy of the mass revealed a moderately differentiated adenocarcinoma (Fig. 4A) invading the subserosa (Fig. 4B). Immunohistochemistry staining revealed homogenous proliferative activity with ki-67 (Fig. 4C) and variable intensity P53 staining (Fig. 4D). The tumor cells were negative for MSH2 and positive for MLH1, PMS2 and MSH6 by immunohistochemistry. The tumor was classified as MSI-High, indicating a high probability of microsatellite instability. Endoscopy revealed innumerable polyps and “coffee ground” blood in the fundus, body, and cardia of the stomach (Fig. 5). The patient’s hospital course was complicated by the development of a rapidly growing colonic abscess that was successfully drained. Genetic testing of the patient via southern blot revealed no abnormalities. However, subsequent gene sequencing analysis of the exons and adjacent intrinsic regions of the APC gene revealed a germline APC mutation 3927del5, resulting in a premature truncation of the APC protein at amino acid position 1312 with the absence of a MYH mutation. A definitive diagnosis of classical familial adenomatous polyposis was made and the patient underwent a total proctocolectomy. Post-surgery, there was no evidence of tumor in 57 nodes reviewed. The patient tolerated the procedure well and plans were made for an ileal-pouch-anal anastomosis. The patient was also started on celoximib 400 mg twice per day to prevent both the recurrence of polyps in the ileal pouch and progression of duodenal adenomas. The patient’s family underwent genetic counseling and although recommended, they refused genetic testing at the time.

Discussion
This case highlights many important genetic and clinical questions regarding a very rare condition. Why did
A de novo germline APC mutation (3927del5) in this patient with no family history of cancer present with a classically autosomal dominant condition? What is the significance of mismatch repair genes and of this patient’s specific genetic mutation? What are the genetic conditions that present similarly to classical FAP? What are the recommended screening procedures and treatments? Can genetic information be further incorporated into treatment?

Mismatch repair genes
Our patient was classified as MSI-High, indicating a high probability of microsatellite instability in her mismatch repair genes. These critical growth regulatory genes are responsible for correcting the nucleotide base mis-pairings that occur during DNA replication.9–12 Several of these genes exist, including human mutS homolog 1, 2, 3, and 6, and human post-meiotic segregation 1 and 2. A tumor is classified as MSI-H when at least two of these genes are affected by instability.13 The prevalence and incidence of microsatellite instability in FAP patients has not been defined. One study suggested that microsatellite instability was only detectable in a small proportion of FAP adenocarcinomas and that these cases may constitute a distinct subgroup among FAP neoplasms.14 The majority of patients with hereditary non-polyposis colorectal cancer (HNPCC), another common hereditary syndrome, have MSI-H tumors, whereas only approximately 15 percent of sporadic tumors are MSI-H. In contrast to microsatellite-stable CRCs, sporadic tumors with...
MSI-H tend to occur in the proximal colon, have a greater mucinous component, contain lymphocytic infiltration, are more frequently poorly differentiated, and are associated with longer survival.

APC gene
Most colon cancers arise when there is an inactivation of the APC gene and the regulation of adenoma epithelial renewal is disrupted. The normal APC protein prevents the accumulation of cytosolic and nuclear beta-catenin by mediating its phosphorylation and degradation. The majority of mutations in the APC gene (both germline and somatic) lead to premature truncation of the APC protein and loss of its beta-catenin regulatory domains. Loss of functional APC results in the nuclear accumulation of beta-catenin, which activates the transcription factor Tcf-4. It is proposed that beta-catenin/Tcf-4 acts as a switch controlling proliferation in the intestinal crypt epithelial cells and the activation of this pathway prevents cell differentiation and induces apoptosis resistance. The genetic events occurring after the APC gene mutation are dependent on the underlying genetic instability—chromosomal instability, germ line mutations in DNA mismatch repair enzymes, and CpG island hypermethylation phenotype [CIMP+].

The process of gene sequencing has become relatively simple with newer technologies. The process begins by breaking chromosomes into shorter pieces. These fragments, differing in length from each other by a single base, are used as templates to be identified. The fragments are then separated by gel electrophoresis and the final base at the end of each fragment is identified, recreating the original sequence of As, Ts, Cs, and Gs for each short piece generated in the first step. Automated sequencers analyze the data and the output consists of a four-color chromatogram showing peaks that represent each of the four DNA bases. After the bases are “read,” computers are used to analyze for errors, gene-coding regions, and other characteristics.

It is hypothesized that new germline APC mutations account for approximately one third of FAP patients who have no family history of the disease. Our patient had a germ line APC mutation 3927del5, resulting in premature truncation of the APC protein at amino acid position 1312. In a review of 417 FAP cases in the literature, the frequency of 3927del5 mutations was 8.9%. In a study by Hadjisavvas et al, the most common APC gene mutation was 2307delA, followed by 1242, and 3927del5. Another study noted that the 3927del5 mutation was associated with a patient presentation of polyposis, but no family history of the disease.

Little is known about the function of specific APC mutations, including 3927del5, and why certain
mutations occur more frequently than others. However, several studies have attempted to correlate specific APC mutations with clinical phenotypes (Table 1). Mutations between codons 169 to 1578 were generally associated with the classic form of FAP. Mutations between codons 1445 and 1578 were associated with desmoid tumors, whereas mutations between codons 279 to 1309 correlated with the development of duodenal polyposis.

Clinical presentation
Classical FAP typically develops in the second or third decade of life and patients generally seek medical attention because of nonspecific abdominal complaints, anemia, or an established family history of FAP. FAP is traditionally diagnosed based on the presence of more than 100 adenomatous colorectal polyps. Genetic testing is now standard for the diagnosis of FAP.

FAP differential diagnosis
Attenuated FAP (AFAP) is a variant of classical FAP and is characterized by a widely variable presentation with fewer colonic adenomas (10 to 100), a lower (up to 80 percent) lifetime risk of colon cancer, a more proximal distribution of adenomas, and a later age of diagnosis of both colonic polyps (average age of 40 to 45) and colon cancer (average age of 50). Consideration of genetic testing for AFAP (APC gene) is recommended in patients with more than 10 to 20 cumulative colonic adenomas.

Table 1. APC mutations and their corresponding phenotypes.

| Mutation                     | Phenotype                                                                 |
|------------------------------|---------------------------------------------------------------------------|
| Between codons 1250 and 1464 | Profuse colorectal polyposis (typically several hundred to thousands of adenomas) |
| Between codons 1445 and 1578 | Desmoid tumors                                                           |
| Between codons 279 to 1309   | Duodenal polyposis                                                        |
| Between codons 463 to 1444   | Retinal lesions (congenital hypertrophy of the retinal pigment epithelium) |
| Downstream from codon 1051   | Severe periampullary lesions                                              |
| Between codon 158 to codon 1596 | AFAP                                                                     |

Approximately 10 to 30 percent of patients with classical FAP, and 90 percent of individuals with attenuated FAP do not have a detectable APC mutation. Many of these individuals have MUTYH associated polyposis (MAP), an autosomal recessive polyposis syndrome caused by biallelic mutations in the MUTYH gene. The two most common MUTYH gene mutations found in MAP in the Caucasian population are Y179C and G396D. The clinical spectrum of MAP is variable, ranging from a clinical phenotype similar to AFAP to one of classic FAP. A large-scale meta-analysis showed that MAP patients demonstrated a 28-fold increase in colon cancer, had a higher frequency of right sided tumors, and had a mean age of 45 at diagnosis. Extra-colonic features associated with MAP include gasto-duodenal polyps, duodenal carcinoma, osteomas, breast cancer in female carriers, congenital hypertrophy of the retinal pigment epithelium, dental cysts, and sebaceous gland tumors. MAP patients are also at increased risk for extra-intestinal cancers including ovarian, bladder, skin, and breast cancer.

Screening and treatment
Fundic gland polyps of the proximal stomach, present in 30 to 100 percent of FAP patients, are thought to have a very low risk of progression to cancer, whereas duodenal polyps, present in 45 to 90 percent of FAP patients, are usually adenomatous and are more likely to progress to cancer. Patients with FAP have an approximately 4 to 12 percent lifetime risk of cancer of the duodenum or papilla of Vater. Adenomas in the distal small bowel and in the stomach occur in 20 to 40 percent of patients with FAP, but have a much lower cancer risk than duodenal adenomas. Cancer has been reported to occur in the ileostomy following colectomy. In a sample of 167 patients who were followed for up to 15 years following colectomy, the risk for developing one or more adenomas in the ileal pouch was 7, 35, and 75 percent at 5, 10, and 15 years follow-up, respectively. FAP patients are also at an increased risk for developing intra-abdominal desmoid tumors and have a lifetime prevalence of 20 percent with a peak incidence around 30 years of age. These tumors are benign, slowly growing fibroblastic neoplasms that tend to recur locally and destroy adjacent vital structures.
Screening of the upper gastrointestinal tract with upper endoscopy for gastric and duodenal polyps has been recommended, although the benefit has not been proven in clinical trials.\textsuperscript{53–55} It is recommended that FAP gene carriers or high-risk family members engage in screening of the lower gastrointestinal tract with a flexible sigmoidoscopy or colonoscopy every 12 months from the age of 10–12 until 35–40 years old if results are persistently negative. Screening recommendations for extra-intestinal lesions in FAP, including hepatoblastoma and thyroid cancer, include serum alpha-fetoprotein testing with abdominal palpation (every six months until the age of six) and annual palpation of the thyroid, respectively.\textsuperscript{56}

Colectomy near the time of initial diagnosis is strongly recommended in patients with multiple large adenomas or adenomas with villous histology and/or high-grade dysplasia. The preferred operation in classical FAP patients is a total proctocolectomy with ileoanal anastomosis. A subtotal colectomy with ongoing surveillance or a total colectomy is reasonable in patients with attenuated adenomatous polyposis who have minor rectal involvement. One study suggested that the specific \textit{APC} genotype may be useful in predicting which patients would be better suited for ileorectal anastomosis versus total proctocolectomy with ileal-pouch-anal anastomosis.\textsuperscript{57} Various medical therapies have been researched as an adjuvant to surgery in order to decrease the overall GI tract polyp burden. Celecoxib, a cyclooxygenase-2 inhibitor, has been shown to reduce the number of colonic polyps (28–44 percent reduction) in adults with FAP.\textsuperscript{58,59} NSAIDs are also being used to slow the development of adenomas prior to colectomy and to delay new polyp formation in the rectum after subtotal colectomy.\textsuperscript{60}

Conclusion
Genetic information has only recently been incorporated into clinical care. Our case of a rare genetic condition highlights the importance of understanding sporadic mutations and how genetic mutations can have widely variable phenotypes. Much more research and randomized clinical trials need to be conducted with FAP patients in order to more definitively characterize random mutations. The hope is that one day it will be possible to risk stratify patients with FAP and improve the screening, diagnosis, and treatment for this condition.

Author Contributions
Conceived and designed the experiments: SZ, NR. Analysed the data: SZ. Wrote the first draft of the manuscript: SZ, NR. Contributed to the writing of the manuscript: SZ, NR. Agree with manuscript results and conclusions: SZ, NR, MC, MF, AH. Jointly developed the structure and arguments for the paper: SZ, NR, MC, MF, AH. Made critical revisions and approved final version: SZ, NR, MC, MF, AH. All authors reviewed and approved of the final manuscript.

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Supplementary Data

A video abstract by the authors of this paper is available. video-abstract10178.mov