Sporadic pediatric severe familial adenomatous polyposis: A case report

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Abstract. Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary precancerous condition caused by germline pathogenetic variants in the tumor suppressor adenomatous polyposis coli (*APC*) gene. Patients with FAP develop multiple gastrointestinal adenomatous polyps usually at the age of ~20 years, which, if untreated, become cancerous in 100% of cases. Genotype-phenotype associations have been extensively described; however, inter- and intra-familial variability exists. It is crucial to characterize the causative pathogenetic variant in each pedigree in order to develop a cancer prevention program and follow-up strategy for at-risk families. The present report describes a severe case of sporadic FAP that was diagnosed when the patient was ~2 years old. The patient was a carrier of the *de novo* pathogenic c.4132 C>T (p.Gln1378X) variant. Additionally, the patient was a carrier of the homozygous c.5465 T>A (p.Asp1822Val) polymorphism, inherited from both parents. However, it remains unclear whether or not this polymorphism is involved in the phenotypic manifestation. This case highlights the need to extend molecular screening to very young children when they show iron-deficiency, anaemia and/or rectal bleeding, even in the absence of a familial history of disease.

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Abbreviations: CRC, colorectal cancer; FAP, familial adenomatous polyposis; APC, adenomatous poliposis coli; EGD, esophagogastroduodenoscopy; SNPs, single nucleotide polymorphisms

Key words: colorectal cancer, pediatric early onset familial adenomatous polyposis, *de novo* germline *APC* gene variants

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women, with 1.4 million estimated cases worldwide, and 700,000 estimated deaths (1,2). Familial adenomatous polyposis (FAP) is an autosomal dominant pre-cancerous syndrome characterized by the development of hundreds to thousands of colorectal adenomatous polyps that, if untreated, lead to CRC in the third to fourth decade of life (3-5). According to the European Medicines Agency, in 2009, there were 3-10/100,000 new cases in the European Union (6).

Germline pathogenic variants in the tumor suppressor adenomatous polyposis coli (*APC)* gene are responsible for FAP (7). *De novo* pathogenetic variants in *APC* are also found in the majority of sporadic cases of FAP (8,9). The *APC* gene is located on chromosome 5q and encodes a 312 kDa protein that is involved in several cellular processes, such as cell migration, adhesion and cell cycle regulation, as well as chromosome segregation, signal transduction and apoptosis (10,11). The tumour suppressing activity of APC depends on its capacity to regulate β-catenin levels in the nucleus. In fact, in the absence of Wnt signalling, APC induces β-catenin degradation. If the extracellular Wnt signal is absent, APC-induced β-catenin degradation is inhibited, β-catenin accumulates in the nucleus and modulates gene transcription. Pathogenic variants that disrupt APC interaction with β-catenin are oncogenic (6-11).

The classical symptoms of FAP, including bleeding, diarrhoea and abdominal pain, are generally diagnosed at around 20-25 years of age. Genetic testing is performed to verify the clinical diagnosis and to identify asymptomatic carriers in affected families. Early FAP detection by genetic screening in at-risk families is crucial in order to implement effective prophylaxis strategies. Moreover, the results of genotyping should be considered together with clinical data to decide the type and time of surgery (12).

Various symptomatic young patients with a family history of FAP have been reported, however we describe a case of severe paediatric FAP caused by a *de novo* pathogenic variant.

Case report

The patient described herein is a ten-year-old boy clinically diagnosed with FAP at the age of nine and recruited to the Ambulatorio di Pediatria of AOU Federico II of Naples in December 2016. The anamnesis revealed that symptoms, i.e. diarrhoea and rectal bleeding, first appeared at the age of two and became more frequent during late childhood. Colonoscopies and esophagogastroduodenoscopy performed in December 2015, December 2016, March 2018 and February 2019 showed hundreds of subcentimetre polyps throughout the colon (Fig. 1A) as well as polyps in the gastric fundus (>10 mm) and body (<5 mm) (Fig. 1B), respectively, thereby confirming the diagnosis of FAP. Histological analysis revealed a *Helicobacter pylori* infection-negative chronic gastritis-like inflammation of the stomach, inflammatory infiltrate in the ileum and low grade dysplasia of the glandular epithelium of the colon. Haematochemical analysis revealed microcytic anaemia with a haemoglobin level of 10.3 g/dl and a mean cellular volume of 66.5fl. The results of physical analysis were unremarkable. As shown in Fig. 2A, there was no family history of FAP, other polyposis syndromes or colon cancer. The patient's parents received clinical and genetic counselling and provided informed consent to molecular screening.

Genomic DNA was extracted from peripheral blood lymphocytes of the proband and his parents as previously described (13). Briefly, 2 ml of the patient's blood were incubated at 37˚ in a red blood lysis buffer (0.15 M NH_4Cl_2 and 0.17 M Tris-HCl, pH 7.65) for 15 min and centrifuged. The lymphocyte pellet was resuspended in a DNA extraction buffer (1 M Tris.HCl, 0.5 M EDTA and 5 M NaCl) and digested with Proteinase K and 10% SDS at 60˚C for ten minutes. After adding 6M NaCl, the sample was centrifuged, the DNA in the supernatant was precipitated with absolute ethanol and resuspended in an appropriate volume of deionized sterile water. The quality and the quantity of the DNA was spectrophotometrically assessed with the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). An absorbance ratio at 260 and 280 nm in the range of 1.8-2.0 was considered good for further analysis.

APC exons 1 to 15 were amplified by polymerase chain reaction (PCR) using 100 ng of the proband genomic DNA and primer pairs described by Groden *et al* (7) in a 50 μl reaction mixture (Table I). The amplification protocol was as follows: 5 min at 95˚C, then 35 cycles of 20 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C, and a final extension of 5 min at 72˚C. All reactions were run in the MyCycler thermal cycler (Bio-Rad). Amplified fragments were run on a 1X agarose gel and visualized with ethidium bromide, then purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's recommendations and subjected to automated Sanger sequencing (Fig. 2B and C).

The sequences analysis was performed by alignment with those present in the GenBank database using the BLASTn software (http://www.ncbi.nlm.nih.gov/blast/html). The accession number of the used reference sequence was NM_000038.4. The same procedure was followed for the genetic analysis of *APC* exon 15 (fragment H) on the DNA of both the patient's parents (Fig. 2B and C).

Genetic counselling excluded a family history of adenomatous polyposis syndrome. Indeed, the patient's parents reported that none of his first grade relatives, including themselves, had symptoms correlated to FAP or to those developed by the proband, such as chronic gastritis-like inflammation of the stomach in the absence of *Helicobacter pylori* infection. However, none of them underwent colonoscopy.

Sequence analysis of the *APC* gene revealed the causative pathogenetic variant in the proband: A heterozygous C to T transition in the exon 15H called c.4132 C>T (p.Gln1378X), which changes the glutamine at codon 1378 in a premature stop codon (Fig. 2C). The proband was also carrier of the rs459552, [c.5465 T>A (p.Asp1822Val)] polymorphism, in fragment L of exon 15 of the APC gene (data not shown). Such polymorphism causes the substitution of a valine with an aspartate at codon 1822 and is reported as benign in the ClinVar database (https://www.ncbi.nlm.nih.gov). Furthermore, the *APC* genetic carrier test for c.4132 C>T (p.Gln1378X) mutation was negative in the proband's parents (Fig. 2C), whereas both carried the rs459552 polymorphism.

Discussion

Approximately 70-80% of FAP cases are caused by inherited pathogenetic variants of the *APC* gene, whereas up to 25% of cases are attributed to *de novo* germline pathogenetic variants (3). Herein, we report the case of a ten-year old boy, clinically diagnosed with severe FAP, in the absence of a family history of polyposis. Genetic analysis of *APC* confirmed the clinical diagnosis. In fact, a stop codon variant, namely c.4132 C>T (p.Gln1378X) in fragment H of exon 15, was identified as the cause of FAP.

Although pathogenetic *APC* germline variants have a penetrance of ~100%, very close genotype-phenotype correlations and marked heterogeneity in the phenotypic expression of FAP are well known. Severe phenotypes, characterized by more than 5,000 polyps and early onset of the disease are associated with pathogenetic variants between codons 1250 and 1464, whereas patients with classical FAP develop hundreds to thousands of adenomatous polyps in the colorectum during the second and third decades of life (14,15). Germline pathogenetic variants between codons 168 and 1680 and deletion of entire *APC* locus are responsible for classical FAP (4,16). Attenuated phenotypes, characterized by a few polyps (~10-100) are associated with pathogenetic variants at the extreme 5' or 3' end of the *APC* gene or in alternatively spliced exon 9. Alterations in the region between codons 1286 and 1513, i.e., the mutation cluster region, probably provide a strong selective advantage to tumour cells. Indeed, the majority of somatic variants and germline *APC* variants are clustered in this region and cause aggressive phenotype (4,16). Thus, in accordance with previous studies, our patient, who carried a pathogenetic truncating variant at codon 1378, had a very early onset of the disease and severe FAP phenotype (17,18). The c.4132 C>T variant is reported as somatic by Liu *et al* (19) and in the *'Catalogue of Somatic Mutations in Cancer (COSMIC)'* (http://cancer.sanger.ac.uk/cosmic/mutation/overview?id=18862). However, Friedl and Aretz (20) found this variant as germlinal in a

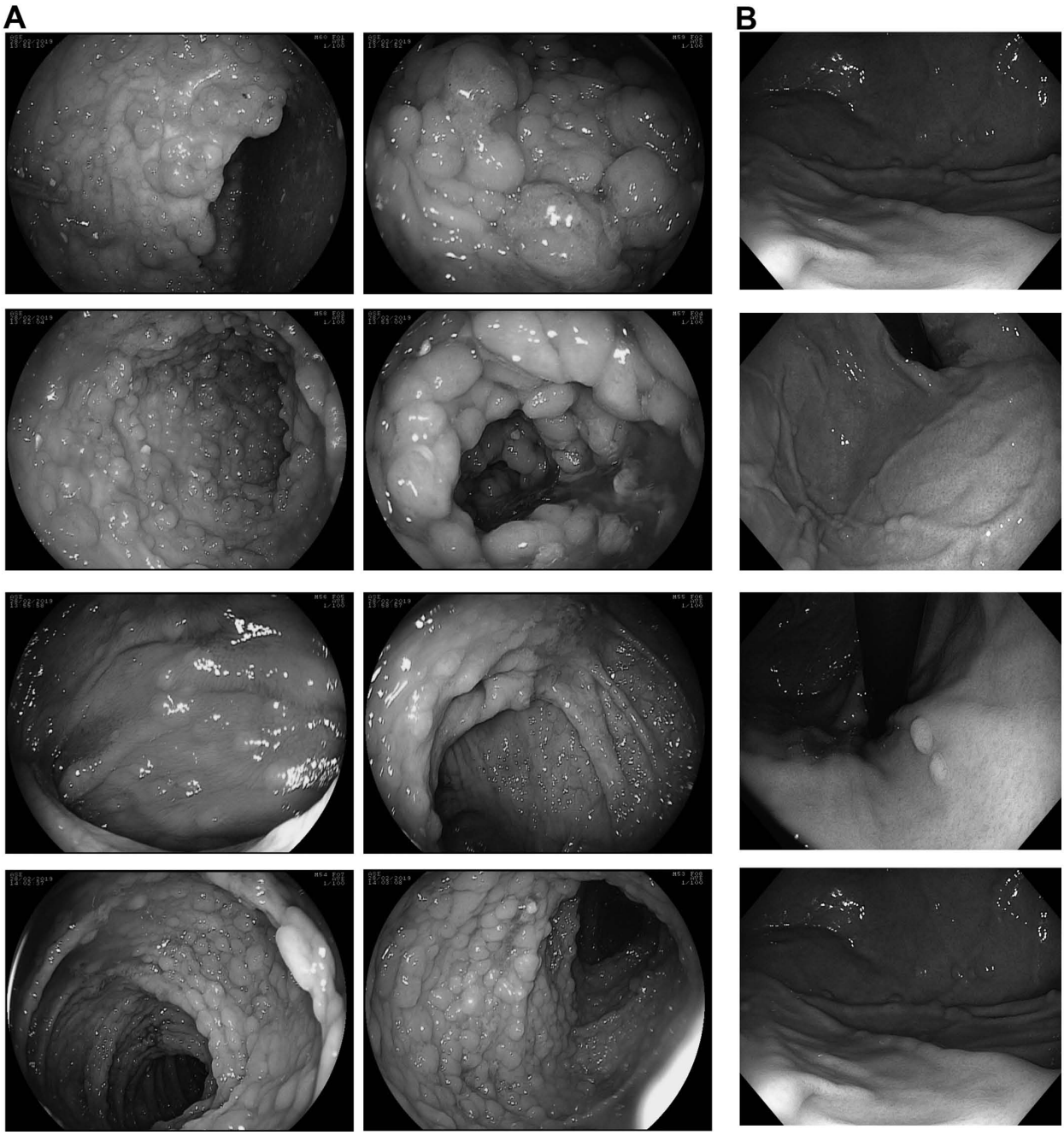

Figure 1. Clinical features of the proband. Colonoscopy and esophagogastroduodenoscopy performed during the last hospitalization in February 2019 revealed evidence of multiple (A) colorectal and (B) gastric adenomatous polyps.

FAP patient, but no information about the patient's history was provided (20). Moreover, to date, no genotype/phenotype correlations have been reported.

The patient also carried a second DNA variant, namely, c.5465 T>A (Asp1822Val). This polymorphism, which was also identified in the patient's parents, causes a valine/aspartate substitution at codon 1822. Several studies have investigated the role of polymorphisms in determining the risk of developing FAP or CRC, hypothesizing that the common variants in CRC genes now considered benign variants are, rather, low-risk alleles.

De Rosa *et al* (21) were the first to identify the Asp1822Val polymorphism. They analysed the genotype of two generations of individuals in ten families and concluded that it was not a disease-causing variant since it was found almost with the same frequency in normal and affected individuals, and it didn't modify the phenotype in any of the FAP patients. Furthermore, according to Fernández-Rozadilla *et al* (22), Asp1822Val is unrelated to CRC development and alleles T and A are equally distributed among cases and controls (P=0.2197) (22). In contrast, in a case-control study in which 1,785 CRC patients and 1,306 controls were analyzed, Picelli *et al* (23) found that the Asp1822Val polymorphism showed an odds ratio slightly lower in subjects carrying CRC than in controls (OR=0.75, CI=0.59-0.94), which suggested that the Asp1822Val substitution could protect from CRC (23). Finally, Wong *et al* (24) evaluated the association between eight *APC* single nucleotide polymorphisms and the risk of developing colorectal adenoma

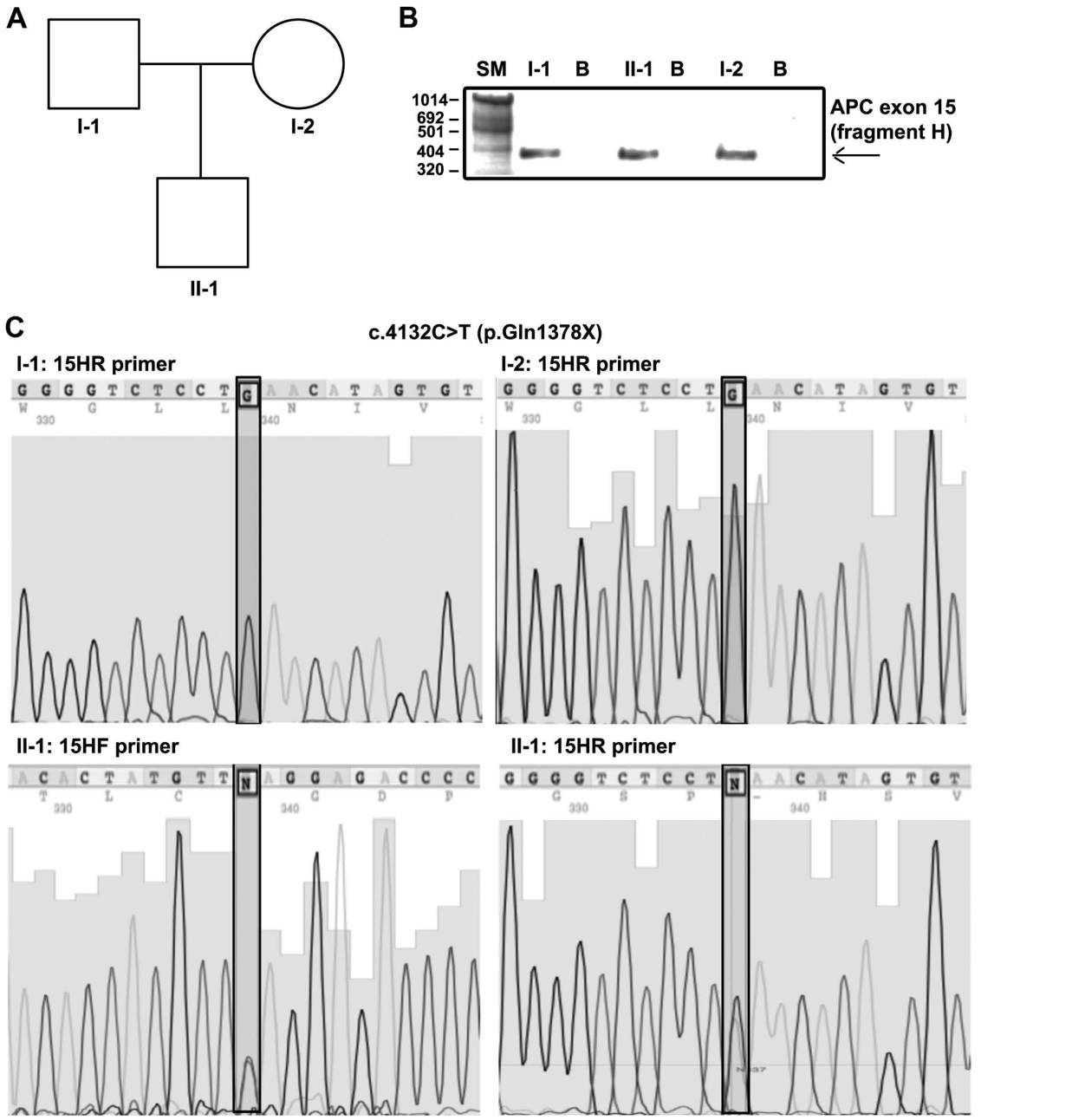

Figure 2. Molecular analysis of c.4132 C>T (p.Gln1378X) variant within the family. (A) Pedigree of the analyzed family. (B) PCR-electrophoresis gel image of exon 15 (fragment H) amplified from the proband's and the proband's parents' genomic DNA and (C) sequence analysis of APC exon 15 (fragment H) performed on fragments amplified from the genomic DNA of the proband (II.1) and his parents (subjects I-1 and I-2). Electropherograms showing the identified variant c.4132 C>T (p.Gln1378X) are reported. Specific nucleotides at position 4132 are shown in black boxes in each electropherogram. APC, adenomatous poliposis coli; SM, DNA size marker; I-1, II-1 and I-2, amplicons obtained by using DNA template from subjects I-1, II-1 and I-2, respectively; B, blank, PCR negative control performed without DNA template.

and concluded that none of the single nucleotide polymorphisms were associated with an increased risk. However, p. Asp1822Val was reported to influence the risk of colorectal adenoma in individuals with higher fat intake.

In conclusion, we found that the germline Gln1378X APC variant is the cause of a very severe sporadic FAP in the proband analyzed in the present study. Notably, the patient had the initial typical symptoms of the disease at the age of two years, although the clinical diagnosis was made when he was nine years old. Young symptomatic FAP patients have been described previously, but rarely in families with negative family history (25). We reported a severe case of FAP with onset in the toddler years, which represents a *de novo* pathogenetic variant (9). In both cases, the very young age at initial presentation (~2 years), as well as the absence of a family history of FAP, delayed the diagnosis for many years. Therefore, we reiterate the importance of endoscopic investigation in a child with iron-deficiency anaemia and a history of rectal bleeding should undergo endoscopic investigation notwithstanding no having a family history of FAP.

Further studies are required to determine whether Gln1378X and p.Val1822Asp play an additive role in his FAP phenotypic manifestations or not.

Table I. Primer pairs used for the genetic analysis of adenomatous poliposis coli exons 1 to 15.

Primers	Forward sequence (5'-3')	Reverse sequence (5'-3')
1 FP/RP	AGGTCCAAGGGTAGCCAAGG	TAAAAATGGATAAACTACAATTAAAAG
2 FP/RP	AAATACAGAATCATGTCTTGAAGT	ACACCTAAAGATGACAATTTGAG
3 FP/RP	TAACTTAGATAGCAGTAATTTCCC	ACAATAAACTGGAGTACACAAGG
4 FP/RP	ATAGGTCATTGCTTCTTGCTGAT	TGAATTTTAATGGATTACCTAGGT
5 FP/RP	CTTTTTTTGCTTTTACTATTAACG	TGTAATTCATTTTATTCCTAATAGCTC
6 FP/RP	GGTAGCCATAGTATGATTATTTCT	CTACCTATTTTTATACCCACAAAC
7 FP/RP	AAGAAAGCCTACACCATTTTTGC	GATCATTCTTAGAACCATCTTGC
8 FP/RP	ACCTATAGTCTAAATTATACCATC	GTCATGGCATTAGTGACCAG
9 FP/RP	AGTCGTAATTTTGTTTCTAAACTC	TGAAGGACTCGGATTTCACGC
9a FP/RP	TCATTCACTCACAGCCTGATGAC	GCTTTGAAACATGCACTACGAT
10 FP/RP	AAACATCATTGCTCTTCAAATAAC	TACCATGATTTAAAAATCCACCAG
10a FP/RP	AGACTAGGACTGAGACATTAATCATC	GGTGAGGAGTGAGAAGAAGGTAATC
11 FP/RP	GATGATTGTCTTTTTCCTCTTGC	CTGAGCTATCTTAAGAAATACATG
12 FP/RP	TTTTAAATGATCCTCTATTCTGTAT	ACAGAGTCAGACCCTGCCTCAAAG
13 FP/RP	TTTCTATTCTTACTGCCTAGCATT	ATACACAGGTAAGAAATTAGGA
14 FP/RP	TAGATGACCCATATTCTGTTTC	CAATTAGGTCTTTTTGAGAGTA
15A FP/RP	GTTACTGCATACACATTGTGAC	GCTTTTTGTTTCCTAACATGAAG
15B FP/RP	AGTACAAGGATGCCAATATTATG	ACTTCTATCTTTTTCAGAACGAG
15C FP/RP	ATTTGAATACTACAGTGTTACCC	CTTGTATTCTAATTTGGCATAAGG
15D FP/RP	CTGCCCATACACATTCAAACAC	TGTTTGGGTCTTGCCATCTT
15E FP/RP	AGTCTTAAATATTCAGATGAGCAG	GTTTCTCTTCATTATATTTTATGCTA
15F FP/RP	AAGCCTACCAATTATAGTGAACG	AGCTGATGACAAGATGATAATG
15G FP/RP	AAGAAACAATACAGACTTATTGTG	ATGAGTGGGGTCTCCTGAAT
15H FP/RP	ATCTCCCTCCAAAAGTGGTGC	TCCATCTGGAGTACTTTCTGTG
15I FP/RP	AGTAAATGCTGCAGTTCAGAGG	CCGTGGCATATCATCCCCC
15J FP/RP	CCCAGACTGCTTCAAAATTACC	GAGCCTCATCTGTACTTCTGA
15K FP/RP	CCCTCCAAATGAGTTACGTGA	TTGTGGTATAGGTTTTACTGGTG
15L FP/RP	ACCCAACAAAAATCAGTTAGATG	GTGGCTGGTAACTTTAGCCTC
15M FP/RP	ATGATGTTGACCTTTCCAGGG	ATTGTGTAACTTTTCATCAGTTGC
15N FP/RP	AAAGACATACCAGACAGAGGG	CTTTTTTGGCATTGCGGAGCT
15O FP/RP	AAGATGACCTGTTGCAGGAATG	GAATCAGACGAAGCTTGTCTAGAT
15P FP/RP	CCATAGTAAGTAGTTTACATCAAG	AAACAGGACTTGTACTGTAGGA
15Q FP/RP	CAGCCCCTTCAAGCAAACATG	GAGGACTTATTCCATTTCTACC
15R FP/RP	CAGTCTCCTGGCCGAAACTC	GTTGACTGGCGTACTAATACAG
15S FP/RP	TGGTAATGGAGCCAATAAAAAGG	TGGGAGTTTTCGCCATCCAC
15T FP/RP	TGTCTCTATCCACACATTCGTC	ATGTTTTTCATCCTCACTTTTTGC
15U FP/RP	GGAGAAGAACTGGAAGTTCATA	TTGAATCTTTAATGTTTGGATTTGC
15V FP/RP	TCTCCCACAGGTAATACTCCC	GCTAGAACTGAATGGGGTACG
15W FP/RP	CAGGACAAAATAATCCTGTCCC	ATTTTCTTAGTTTCATTCTTCCTC

FP, forward primer; RP, reverse primer.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MDR and PI designed the study. MDR, AC, AA and FC performed genetic analysis. EM and MR provided sample

collection and clinical support. MDR and PI contributed to data interpretation. MDR and AC wrote the manuscript, and FD and RL critically revised the manuscript and participated in the analysis and interpretation of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent has been provided by the proband's parents. All methods are part of the clinical practice necessary to carry out the molecular analysis of the APC gene, requested by the proband's parents. Furthermore, the proband's parents agree that the DNA sample no longer needed for the study is used for medical research purposes in an anonymous form and/or in epidemiological cases. The procedures reported in this study were performed in accordance with the rules of the Good Clinical Practice Guidelines (GCP) and the ethical principles set out in the Declaration of Helsinki. The study was also authorized by 'Comitato etico per le attività Biomediche-Carlo Romano of the University of Naples Federico II' (protocol no. 35/17).

Patient consent for publication

Not applicable. All identifying information, case details, personal information or images that may enable an individual to be identified, are not included in the text.

Competing interests

The authors declare that they have no competing interests.

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