Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Atypical clinical presentations of APC-associated polyposis linked to pathogenic variants c.1333C>T p.(Gln445Ter) and c.221-2A>G p.(?) – two case reports

**Feyza Canan**
Cedars-Sinai Medical Center
**Gabriela Felix**
Cedars-Sinai Medical Center
**Rocio Alvarez**
Cedars-Sinai Medical Center
**Kevin Tsang**
Cedars-Sinai Medical Center
**Hongyu Liu**
Cedars-Sinai Medical Center
**Olivia Tan**
Cedars-Sinai Medical Center
**John Lee**
Cedars-Sinai Medical Center
**Zuri Murrell**
Cedars-Sinai Medical Center
**Daniel M. Kim**
Cedars-Sinai Medical Center
**Nicholas Tatonetti**
Cedars-Sinai Medical Center
**Jun Gong**
Cedars-Sinai Medical Center
**Andrew E. Hendifar**
Cedars-Sinai Medical Center
**Megan P. Hitchins**
megan.hitchins@moffitt.org

Cedars-Sinai Medical Center

**Case Report**

**Keywords:** Familial adenomatous polyposis, attenuated polyposis, APC

**Posted Date:** November 12th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11485234/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

## Abstract

# Background

Different germline pathogenic variants in the adenomatous polyposis coli (*APC*) gene can confer mild to severe presentations of familial adenomatous polyposis (FAP) with phenotypes ranging from attenuated FAP with multiple polyps to florid polyposis with high risk for colorectal cancer (CRC) development, often at a young age. FAP can be accompanied by extra-colonic manifestations, including desmoid and other tumors. We report two index cases with atypical clinical presentations and distinct disease trajectories associated with two rare germline pathogenic variants of *APC*.

# Case presentation:

Patient 1 was an African American male who presented with increasingly high polyposis burden between the ages of 31 and 33 years and rectal cancer at 33 years. He also had a history of cranial fasciitis in infancy and dermal cysts. Germline genetic testing revealed the *APC* c.1333C > T p.(Gln445Ter) nonsense variant. He had no family history of polyposis or CRC but did have a family history of cranial fasciitis. His family declined cascade genetic testing. Exome sequencing revealed additional germline heterozygous pathogenic variants in *NBAS* and *RXYLT1* that were considered unlikely to have contributed to his phenotype. Multiple somatic mutations in *APC* and other CRC-associated driver genes were identified in the rectal tumor, consistent with polyclonal origins in carcinogenesis. Patient 2 is a European female with a family history of serial and multiple polyps who developed multiple polyps over a decade of follow-up. Germline genetic testing revealed the *APC* c.221-2A > G splice variant in her and first-degree relatives. RNA analyses in her normal tissues and polyps revealed distinct aberrantly spliced transcripts, which were outnumbered by normal transcripts in normal tissues, providing one possible explanation for her milder phenotype, and enriched in polyps.

# Conclusions

These case reports illustrate the spectrum and complexity of genotype-phenotype correlations observed among *APC* pathogenic variants and the importance of comprehensively integrating clinical, family history, genetic, and functional information when evaluating individualized risk and surveillance strategies.

## Background

Familial adenomatous polyposis (FAP) is characterized by hundreds to thousands of colorectal adenomatous polyps that, if left untreated, will inevitably develop into colorectal carcinoma (CRC) with a mean age of 39 years. [1] This is commonly accompanied by extracolonic manifestations, which can include congenital hypertrophic retinal pigment-epithelium lesions (CHRPEs), and ectodermal and

mesodermal tumors including desmoid fibromatoses, osteomas, epidermoid and sebaceous cysts, and upper gastrointestinal polyps. FAP is autosomal dominant, caused by heterozygous germline pathogenic variants (PV) within the adenomatous polyposis coli (*APC*) gene, which are most frequently protein-truncating, including nonsense or frameshift mutations. [2] Germline PV in *APC* arise *de novo* in 20%-30% of FAP cases, who present with no remarkable family history. [3]

There is variable clinical expressivity, both in the severity of polyposis, as well as the presentation of extracolonic manifestations. The phenotypically milder attenuated FAP (AFAP) is defined by a reduced polyp burden (< 100) and lifetime risk for CRC (~ 70%), delayed age of CRC onset, typically above 40 years, and less frequent extracolonic manifestations. [3] Genotype-phenotype correlations with *APC* PV have been reported, with AFAP more frequently associated with germline PVs located towards the 5' end of the gene (in the first five exons), within alternatively spliced exon 9 region, or in the distal 3' region. [4, 5] Since the APC protein functions as a homodimer, highly-penetrant PVs may act in a dominant-negative fashion, resulting in about three-quarters of homodimers being malfunctional mutant-mutant or mutant-wildtype protein homodimers. PVs at the 5' end result in very short, truncated proteins that may fail to form homodimers at all, which may account for the less severe phenotype. [6] Other PVs may result in the production of reduced levels of aberrant transcripts or these may be subject to nonsense-mediated mRNA decay. [3] Patients with AFAP, or those described as having developed multiple polyps over their life-times, with negative germline genetic test results have been identified with mosaic PVs of *APC* in their colon tissues, which presents a molecular diagnostic challenge. [7, 8]

Herein, we report two cases that illustrate the diverse clinical manifestations associated with germline PVs of the *APC* gene. The first patient is an African American male with cranial fasciitis in infancy who was diagnosed with classic FAP and early-onset rectal cancer in the absence of any family history of polyposis or CRC. Germline genetic testing revealed the *APC* c.1333C > T p.(Gln445Ter) nonsense PV. The second patient is a female of Northern European heritage harboring the acceptor splice-site variant *APC* c.221-2A > G p.(?) with a personal and family history of multiple polyps. Through RNA analyses, we show that this variant produces distinct erroneously spliced transcripts, although these are outnumbered by wild-type transcripts in normal tissues.

## Case Presentations

# Patient 1 (P1) Presentation

P1 was an African American male with no family history of polyposis or CRC (Fig. 1A), who at the age of 31 years, was identified with “hundreds of diffuse polyps” throughout the colon during a baseline colonoscopy conducted following presentation with symptoms of severe anemia, altered bowel habits, hematochezia, and exercise intolerance. Upper endoscopy to the duodenum revealed antral gastritis that was positive for *Helicobacter pylori*, but no polyps. At age 33 years, follow-up colonoscopy and upper endoscopy showed marked disease progression with extensive polyposis consistent with FAP and persistent gastritis. Systematic biopsies from six colonic segments were all histologically identified as

tubular adenomas. The patient was referred to a tertiary care center and underwent genetic counseling for suspicion of FAP. Germline genetic testing (ColoNext multigene panel, Ambry Genetics) identified *APC* c.1333C > T p.(Gln445Ter), a nonsense PV. This variant is rare, with a minor allele frequency (MAF) of 0.00002 observed in African populations and no other ancestral group in gnomADv4 (most recently accessed May 20, 2026). *APC* c.1333C > T p.(Gln445Ter) has previously been reported in the literature in one index case with polyposis and a positive family history of cancer from Turkey, [9] although further details were not provided (Supplementary Table 1). With this genetic confirmation of FAP, P1 underwent a total proctocolectomy with ileoanal anastomosis and laparoscopic rectal mucosectomy at age 33 years. Pathology revealed a moderately differentiated adenocarcinoma of the rectum (AJCC stage IIIb), along with innumerable tubular, tubulovillous, and villous adenomas, including one with high-grade dysplasia. P1 underwent adjuvant chemotherapy, but subsequently recurred with mesenteric, pelvic, and liver metastases. P1 ultimately died at the age of 38 years following disease progression and multiple infections.

P1 consented to the study and provided a blood sample, though notably, he had metastatic disease and had already undergone multiple cycles of chemotherapy by the time of this research blood draw. A three-generation pedigree was assembled in consultation with P1 and his parents (Fig. 1). There was no family history of polyposis or CRC; both parents had undergone colonoscopies most recently in their sixties with no remarkable findings and a younger brother (who had not undergone colonoscopy) was asymptomatic at 30 years. The first-degree relatives (FDRs) declined cascade genetic testing for the *APC* c.1333C > T p.(Gln445Ter) PV. Past medical records for P1 documented cranial fasciitis in infancy, which was surgically excised, and three dermal cysts (two excised). The histology of these lesions was not recorded and no samples were available for study. However, a family history of cranial fasciitis was reported in two male relatives on the paternal side (Fig. 1A).

Research-based whole-exome sequencing (WES) was conducted in DNA extracted from “buffy coat” (centrifuged circulating white blood cells), hereafter referred to as peripheral blood lymphocytes (PBL), and paired samples of pre-treatment FFPE tumor and adjacent normal colon tissues. The same *APC* c.1333C > T p.(Gln445Ter) variant and two additional germline heterozygous PVs, one a nonsense variant in *NBAS* (ENST00000281513.10) c.4336C > T p.(Arg1446Ter), the other a frameshift variant resulting in premature protein truncation in *RXYLT1* (formerly *TMEM*) (NM_014254.3) c.515_519del p.(Ala172AspfsTer31), were identified in WES of PBL DNA. Biallelic PVs in these two genes are associated with distinct autosomal recessive syndromes. *NBAS* PVs cause a multisystem phenotype with variable presentations including acute liver failure, short stature, optic atrophy, atypical osteogenesis imperfecta, craniofacial dysmorphologies and asymmetry, skeletal malformations, anemia, and immune system dysfunction. *RXYLT1* PVs cause Walker-Warburg syndrome or the lesser muscle-eye-brain syndrome featuring alpha-dystroglycanopathies and severe cobblestone lissencephaly, and intellectual disability. While these findings provide a more comprehensive molecular profile of P1, these specific variants are unlikely to have contributed to his cancer development. However, it is possible that in heterozygous form, they may have contributed to, or exacerbated, clinical conditions such as anemia, or perturbed liver function or immune responses while receiving treatment.

Cranial fasciitis is a rare, myofibroproliferative lesion of the scalp and bone beneath and can comprise mixed histologies. Various genomic rearrangements involving *USP6* that result in ectopically over-expressed *USP6* fusion transcripts have been shown to be a recurrent event in sporadic tumors. [10] However, cranial fasciitis with aberrant nuclear β-catenin immunohistochemical staining has previously been reported in pediatric cases with inherited PVs in *APC*, as well as sporadic lesions with other mechanisms of dysregulated Wnt/ β-catenin signaling. [11, 12] One report describes an infant with a germline truncating *APC* mutation (c.878delG) who developed cranial fasciitis and a somatic nonsense mutation (c.4132C > T) on the other allele. [11] An earlier report documented a 2-year-old case with a cranial tumor exhibiting overlapping features of both cranial fasciitis and desmoid tumors in the context of a paternal history of FAP. The tumor harbored the paternally inherited germline truncating PV, *APC* c.3372insT p.(Glu1124fs), and a somatic deletion of the maternal wild-type allele. [12] Although cranial fasciitis is generally considered to be sporadic, the paternal family history in P1 suggests a heritable component. The absence of any remarkable family history of polyposis or CRC in either parent or prior generations in the family of P1 raises the question of whether the PV arose *de novo* in him. Yet, if the cranial fasciitis is indeed linked to the *APC* c.1333C > T p. (Gln445Ter) PV in him, then this would suggest other relatives with cranial fasciitis may also have been carriers of this *APC* PV, in which scenario there must be a lack of penetrance for polyposis and CRC in other family members who carried/carry this variant. Alternatively, a genetic variant other than *APC* segregating in the family could underlie this phenotype. While biallelic PVs of *NBAS* cause skeletal and craniofacial dysplasia, this gene has not been associated with cranial fasciitis, and neither has *RXYLT1*.

Somatic analyses of WES data identified additional protein-altering variants in *APC* and other CRC-associated genes, in the primary rectal tumor. Interestingly, overlapping somatic variants and a distinct somatic *BRAF* mutation were also identified in the “PBL” DNA sample, which may have been derived from circulating tumor cells within the “buffy coat” of the blood sample, given this was drawn after P1 had developed metastatic disease (Fig. 1B). A complete list of variants, including their origin (PBL and/or tumor) and sharing status, is provided in Supplementary Table 2. Somatic variants included multiple distinct loss-of-function (LOF) mutations in *APC* as likely “second hits” in tumorigenesis, distinct LOF variants in other tumor suppressor genes and DNA repair genes, as well as gain-of-function mutations in *KRAS*, *NRAS*, and *NBAS*, consistent with polyclonality previously observed in FAP-associated carcinogenesis. [13]

## Patient 2 (P2) Presentation

P2 (Fig. 2A) is a White female propositus of Northern European heritage who was asymptomatic at the age of 38 years but concerned about cancer prevention due to the finding on cascade genetic testing that she bore the *APC* c.221-2A > G (p?) variant. This variant had previously been identified in her mother, who had been diagnosed with multiple polyps (estimated 50 in total) during prior screening colonoscopies, until at the age of 57 years, she was recommended to undergo germline genetic testing for predisposition to polyposis and CRC. Cascade testing in the propositus and her two brothers through

Color showed all three siblings were positive for *APC* c.221-2A > G (p?) (Fig. 2A). Her elder brother underwent a baseline colonoscopy at the age of 30 years, during which four gastric polyps and six colonic polyps were identified. Her youngest brother has had just one polyp identified at baseline colonoscopy aged 27 years (Fig. 2A).

*APC* c.221-2A > G has been classified as pathogenic or likely pathogenic for FAP or AFAP in ClinVar (most recently accessed July 1, 2026), with reports in two other index cases (Supplementary Table 3). [14, 15] One female patient who underwent colonoscopy due to a family history of CRC and duodenal cancer that included early-onset CRC in two siblings, was diagnosed with rectal cancer at 34 years and developed multiple polyps 10 years thereafter. [14] The other case reported polyposis without CRC, but no further details. [15] This variant is extremely rare, with the highest MAF of 0.00003 in the African population and 0.0000057 in the European population within gnomAD v4 (most recently accessed May 20, 2026). The c.221-2A > G (p?) variant is located within the 5' acceptor splice site in intron 3, two bases upstream of exon 4. *APC* c.221-2A > G was previously predicted by *in silico* splice tools to result in altered splice transcripts due to the activation of pseudo acceptor splice sites located more distally within exon 4 that would ultimately produce frameshift transcripts. [14]

P2 consented to the study and provided a blood sample two months prior to undergoing a baseline colonoscopy and upper endoscopy. *APC* c.221-2A > G was detected in-house by Sanger sequencing of PBL DNA (Fig. 2B). Prior approval from the institutional review board (IRB), patient, and gastroenterologists were granted to obtain a fresh pinch biopsy sample of normal colonic mucosa (NCM) and any of the smallest polyp(s) deemed supernumerary to requirements for clinical diagnostic purposes during the procedures for research. A total of 14 colonic polyps were identified and resected. Two small polyps (≤ 3 mm diameter) and pinch biopsy of NCM tissue were obtained and stored in RNA-Later for RNA analyses. To functionally validate the predicted aberrant splicing, we performed targeted, clonal cDNA sequencing of reverse-transcriptase PCR products generated across exons 2–5 from the two polyps, adjacent NCM tissue, and peripheral blood mononuclear cells (PBMCs) from P2, and PBLs from a colonoscopy-verified healthy control matched by race, sex, and age (± 5 years). Three aberrant splice transcripts were observed only in P2 samples (Fig. 2C). The most abundant of these had the first two nucleotides (AG) of exon 4 deleted due to the utilization of a pseudo-acceptor splice site. This “delAG” transcript was predicted to result in a frameshift, introduction of a premature stop codon, and a truncated protein. The second aberrant transcript had a deletion of the first 12 bases (AGCTTAACTTAG) of exon 4, again due to the activation of a slightly more distal pseudo-acceptor splice site. This was predicted to result in an in-frame deletion of four amino acids. The third showed skipping of exon 3, which was predicted to result in a frameshift and truncated protein. The “delAG” transcript was about 7-12-fold more abundant than each of the other two aberrant transcripts, indicating utilization of the first pseudo-acceptor splice site as an alternative appeared to be the predominant aberrant splicing activity. Whether the variant *APC* c.221-2A > G allele produced any normal transcripts could not be directly determined in the mRNA of P2 due to the lack of any additional heterozygous (exonic) variants (such as SNPs) with which to distinguish the allelic origins of normal transcripts. However, enumeration of wild-type and total abnormal transcripts (pooled) in each tissue source from P2 showed the ratio of wild-type

exceeded abnormal transcripts in both NCM (1.4:1) and PBMCs (1.9:1). This suggests that in normal tissues the variant allele also produced wild-type transcripts. By contrast, the ratio of wild-type to total abnormal transcripts was reduced in both polyps (1:5 and 1:10). Whether the erroneously spliced transcripts were amplified in polyps due to loss-of-heterozygosity of the wild-type allele as somatic second-hits, or due to other mechanisms of allelic expression imbalance, could not be tested due to sample insufficiency.

In follow-up with P2 eight years later, at the age of 46 years, she reported that she and her family had been clinically managed by 1–2 yearly colonoscopies and biennial upper endoscopies with resections of multiple polyps from each family member when detected, but none have gone on to develop CRC. The most recent colonoscopy and pathology report for P2 at age 45 years reported nine sessile polyps and seven tubular adenomas spread throughout the colon and negative findings in upper endoscopies. Her mother has continued to undergo colonoscopies at less frequent time intervals, with multiple “increasing numbers of” polyps identified each time and an advanced adenoma of 3 cm identified at age 65 years. No clinical manifestations outside the gastrointestinal tract have been identified. The father, who has not undergone genetic testing, underwent his first colonoscopy at 71 years of age, in which 11 polyps were identified. The phenotype among proven *APC* c.221-2A > G heterozygotes in this family thus ranges from serial single polyps beginning at age 27 years to multiple polyps from the age of 30 years, with increasing polyp burden thereafter, consistent with AFAP.

## Discussion and Conclusions

We investigated two cases with PVs in *APC* that are recurrent (previously identified), yet rare in MAF, coupled with distinct clinical phenotypes. P1, who carried the *APC* c.1333C > T p.(Gln445Ter) PV, displayed classic FAP with early-onset rectal cancer plus extracolonic features described as “dermal cysts” and infant cranial fasciitis in medical records. Whether cranial fasciitis was a manifestation of his FAP remains unclear, given there was a family history of this among second-degree relatives but no family history at all of polyposis or CRC. Limitations of study in this family were that relatives of P1 did not undergo cascade genetic testing, reports of cranial fasciitis in the pedigree could not be confirmed through medical records, and the cranial fasciitis lesion from P1 was unavailable for study to determine if this was linked to *APC*.

P2, her mother and siblings, who carried the splice variant *APC* c.221-2A > G p.(?), all displayed an AFAP phenotype. While two other index cases have been reported, one including a family featuring early-onset CRCs with multiple polyps described a decade later in the proband, the other was not well described other than as having polyposis.[14, 15] While no prior functional testing of the impact of this variant had been conducted in them, *in silico* predictions suggested altered splicing. Our functional studies in P2 confirmed and extended these predictions by demonstrating the variability in erroneous splicing, with the predominant altered transcript being produced by activation of the first of two pseudo splice acceptor sites within exon 4. We found the wild-type transcripts exceeded aberrantly spliced transcripts in normal tissues, providing a plausible explanation for the milder phenotype. This may have been due to the

production of a proportion of normal transcripts from the variant allele – it is generally considered common for splice variants to produce some wild-type transcripts. Enrichment of aberrantly spliced transcripts was observed in the adenomatous polyps. This illustrates the complexity of splice variants and how their behavior can be influenced by surrounding sequence context. Splice mutations are comparatively rare in FAP, comprising under 10% of all *APC* mutations. [2] This frequency may be skewed by ascertainment bias if they confer a milder phenotype.

These findings underscore the importance of integrating clinical, including family history, with comprehensive genetic and functional evaluation, when assessing individuals and families with the hereditary high-risk condition associated with *APC* pathogenic variants. Continued reporting of rare variants will aid ongoing refinements of genotype–phenotype correlations and inform individualized risk stratification and surveillance strategies in those at risk.

# Methods

This study was approved by the Cedars-Sinai Medical Center Institutional Review Board under protocol Pro00049624. Participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki. Consent included permission to publish in de-identified format.

Patients 1 and 2 were ascertained via consultations with specialists in high-risk genetics and gastroenterology in 2017. Peripheral blood was collected from both patients by venipuncture. Genomic DNA was extracted from “buffy coat” after standard centrifugation to enrich for peripheral blood leukocytes using the QIAGEN Blood Mini Kit according to the manufacturer’s protocol. For Patient 1, extracted DNA was submitted for whole exome sequencing, and tumor tissue was additionally evaluated for mismatch repair (MMR) status by immunohistochemistry. WES Libraries were prepared using Illumina DNA Prep with Exome 2.5 Enrichment (Illumina, USA). Impact classification was assigned using Ensembl Variant Effect Predictor (VEP). High-impact variants included frameshift, stop-gained, and essential splice-site alterations predicted to strongly disrupt protein function, whereas Moderate-impact variants primarily comprised missense substitutions predicted to alter amino acid sequence without causing protein truncation.

For Patient 2, the splice-site variant was confirmed by PCR amplification followed by bidirectional Sanger sequencing of the purified amplicon (primer sequences provided in Supplementary Table 4). To characterize the effect of the splice-site variant at the transcript level, RNA was obtained from NCM via pinch-biopsy and two small (< 3 mm diameter) endoscopically resected colorectal polyps preserved in RNALater, obtained during a baseline interventional colonoscopy and PBMCs. Messenger RNA was isolated, reverse transcribed to complementary DNA (cDNA), and targeted RT-PCR amplification of the *APC* transcript was performed between exons 2 and 5 (primer sequences provided in Supplementary Table 4). RT-PCR products were purified, cloned using the Promega pGEMTeasy plasmid system, and 24–48 individual colonies per tissue type were picked for plasmid extraction. Sanger sequencing of the inserts was performed using SP6 plasmid primers to identify individual transcripts (GeneWiz). Trimming

of vector sequences and analysis of inserts was performed using LaserGene software (DNAStar, Madison, USA.

## Abbreviations

AFAP
Attenuated familial adenomatous polyposis
APC
Adenomatous polyposis coli
CF
Cranial fasciitis
CHRPE
Congenital hypertrophy of retinal pigment epithelium
CRC
Colorectal cancer
FAP
Familial adenomatous polyposis
PV
Pathogenic variant

## Declarations

**Ethics approval and consent to participate:**

This study was approved by the Cedars-Sinai Institutional Review Board under protocol Pro00049624. All consent forms were reviewed and approved by the IRB. All participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki.

**Consent for publication:**

All participants provided written informed consent for the publication of de-identified data derived from this study. No personal identifiers are included, and all data have been anonymized to ensure confidentiality.

# Competing interests:

J.G. reports receiving consulting fees and honoraria from the following companies: EMD Serono, Exelixis, Natera, Eisai, Janssen, Pfizer, Bayer, Taiho, Agenus, Seagen, and Caper Labs. The remaining authors declare that they have no competing interests.

# Funding:

This study was funded by the Cedars-Sinai Precision Health Initiative Award (MH, AH). Cedars-Sinai Medical Center Biomedical Data Science Shared Resources Educational Voucher Program (GF), the Cedars-Sinai Cancer Development Fund (MH).

# Author Contribution

Feyza Canan and Gabriela Felix contributed equally to this work.Study design and conception: MH, AHSamples collection and resources: MH, JG, AHFinancial resources: MH, AHData acquisition: All authorsData analysis and interpretation: All authorsManuscript first draft writing: FC, GF, MHManuscript review and approval: All authors

# Acknowledgements:

Not applicable

# Data Availability

Availability of data and materials: Analyses were performed using publicly available bioinformatics software with documented workflows and cited versions, following standard command-line usage as described in the Supplemental Methods. No custom algorithms were developed. De-identified data generated in this study are available from the corresponding authors upon reasonable request. No custom code was generated in this study.

## References

1. Leoz ML, Carballal S, Moreira L, Ocaña T, Balaguer F. The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. Appl Clin Genet. 2015;8:95–107. 10.2147/TACG.S51484.
2. Kerr SE, Thomas CB, Thibodeau SN, Ferber MJ, Halling KC. APC germline mutations in individuals being evaluated for familial adenomatous polyposis: a review of the Mayo Clinic experience with 1591 consecutive tests. J Mol Diagn. 2013;15(1):31–43. 10.1016/j.jmoldx.2012.07.005.
3. Knudsen AL, Bisgaard ML, Bülow S. Attenuated familial adenomatous polyposis (AFAP): a review of the literature. Fam Cancer. 2003;2(1):43–55. 10.1023/A:1023286520725.
4. Sieber OM, Segditsas S, Knudsen AL, Zhang J, Luz J, Rowan AJ, et al. Disease severity and genetic pathways in attenuated familial adenomatous polyposis vary greatly but depend on the site of the germline mutation. Gut. 2006;55(10):1440–8. 10.1136/gut.2005.087106.
5. Soravia C, Berk T, Madlensky L, Mitri A, Cheng H, Gallinger S, et al. Genotype-phenotype correlations in attenuated adenomatous polyposis coli. Am J Hum Genet. 1998;62(6):1290–301. 10.1086/301883.

6. Dobbie Z, Spycher M, Hurliman R, Ammann R, Ammann T, Roth J, et al. Mutational analysis of the first 14 exons of the adenomatous polyposis coli (APC) gene. Eur J Cancer. 1994;30A(11):1709–13. 10.1016/0959-8049(94)00294-F.
7. Terlouw D, Suerink M, Buitelaar Y, van Leerdam ME, Hes FJ, van Egmond D, et al. Prevalence and consequences of APC mosaicism in patients with colorectal adenomas. Gastroenterology. 2026;170(5):932–41. 10.1053/j.gastro.2025.10.028.
8. Jansen AML, Crobach S, Geurts-Giele WRR, van den Akker BEWM, Garcia MV, Ruano D, et al. Distinct patterns of somatic mosaicism in the APC gene in neoplasms from patients with unexplained adenomatous polyposis. Gastroenterology. 2017;152(3):546–e5493. 10.1053/j.gastro.2016.10.040.
9. Ercoskun P, Yuce Kahraman C, Ozkan G, Tatar A. Genetic characterization of hereditary cancer syndromes based on targeted next-generation sequencing. Mol Syndromol. 2022;13(2):123–31. 10.1159/000518927.
10. Paulson VA, Stojanov IA, Wasman JK, Restrepo T, Cano S, Plunkitt J, et al. Recurrent and novel USP6 fusions in cranial fasciitis identified by targeted RNA sequencing. Mod Pathol. 2020;33(5):775–80. 10.1038/s41379-019-0422-6.
11. Rakheja D, Cunningham JC, Mitui M, Patel AS, Tomlinson GE, Weinberg AG. A subset of cranial fasciitis is associated with dysregulation of the Wnt/β-catenin pathway. Mod Pathol. 2008;21(11):1330–6. 10.1038/modpathol.2008.112.
12. de Silva DC, Wright MF, Stevenson DAJ, Clark C, Gray ES, Holmes JD, et al. Cranial desmoid tumor associated with homozygous inactivation of the adenomatous polyposis coli gene in a 2-year-old girl with familial adenomatous polyposis. Cancer. 1996;77(5):972–6. 10.1002/(SICI)1097-0142(19960301)77:5%3C972::AID-CNCR25%3E3.0.CO;2-#.
13. Van Egeren D, Schenck RO, Khan A, Horning AM, Mo S, Weiss CL, et al. Polyclonal origins of human premalignant colorectal lesions. Nature. 2026;650(8103):1017–24. 10.1038/s41586-025-09930-y.
14. Beaton MD, Taylor B, Driman D, Ainsworth P, Adams PC. Colonic interposition in a woman with attenuated familial adenomatosis polyposis: does the location of the colon affect polyp formation? Can J Gastroenterol. 2008;22(7):634–6. 10.1155/2008/780204.
15. Poliani L, Greco L, Barile M, Dal Buono A, Bianchi P, Basso G, et al. Canonical and uncanonical pathogenic germline variants in colorectal cancer patients by next-generation sequencing in a European referral center. ESMO Open. 2022;7(6):100607. 10.1016/j.esmoop.2022.100607.

# Figures

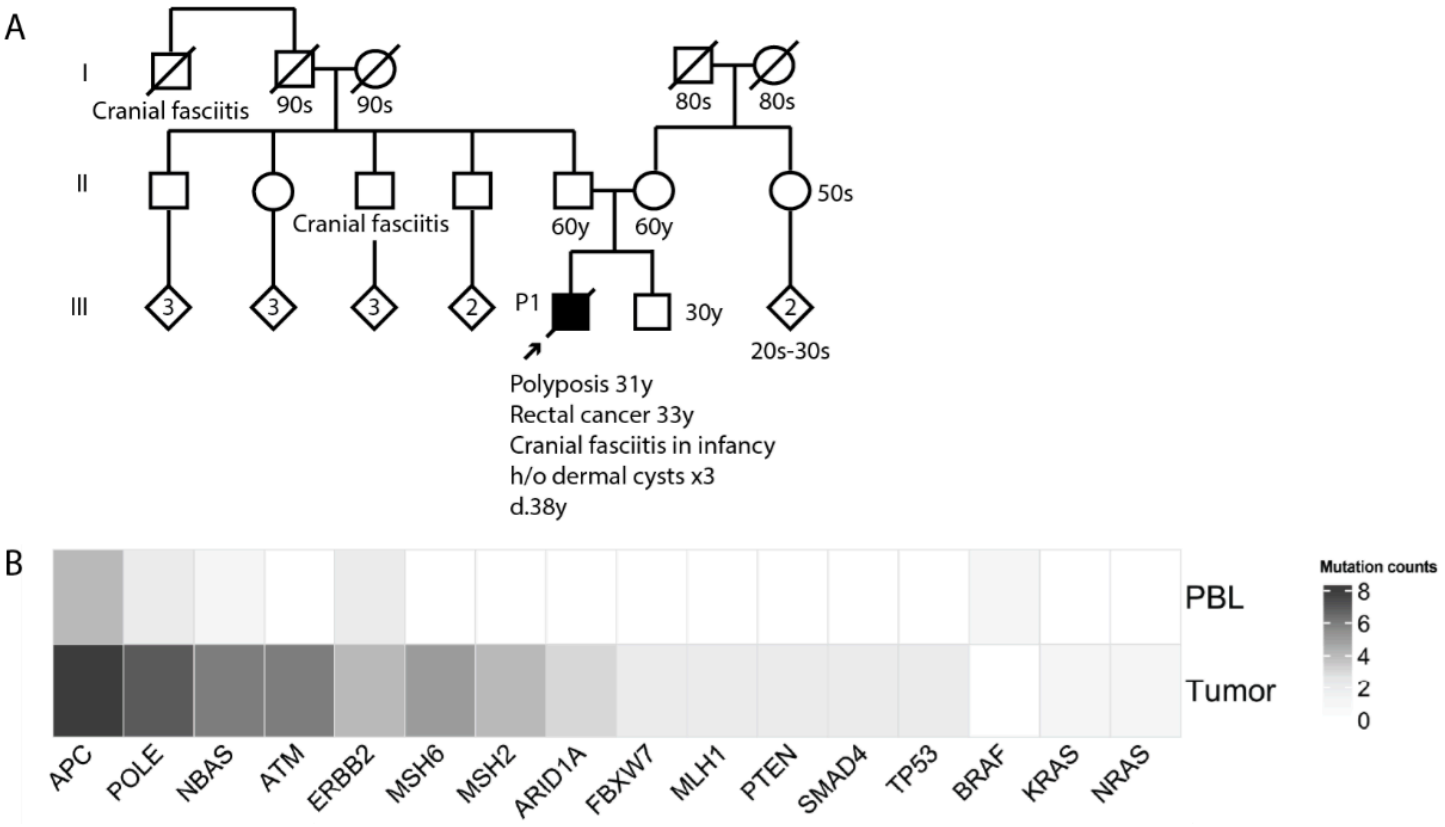
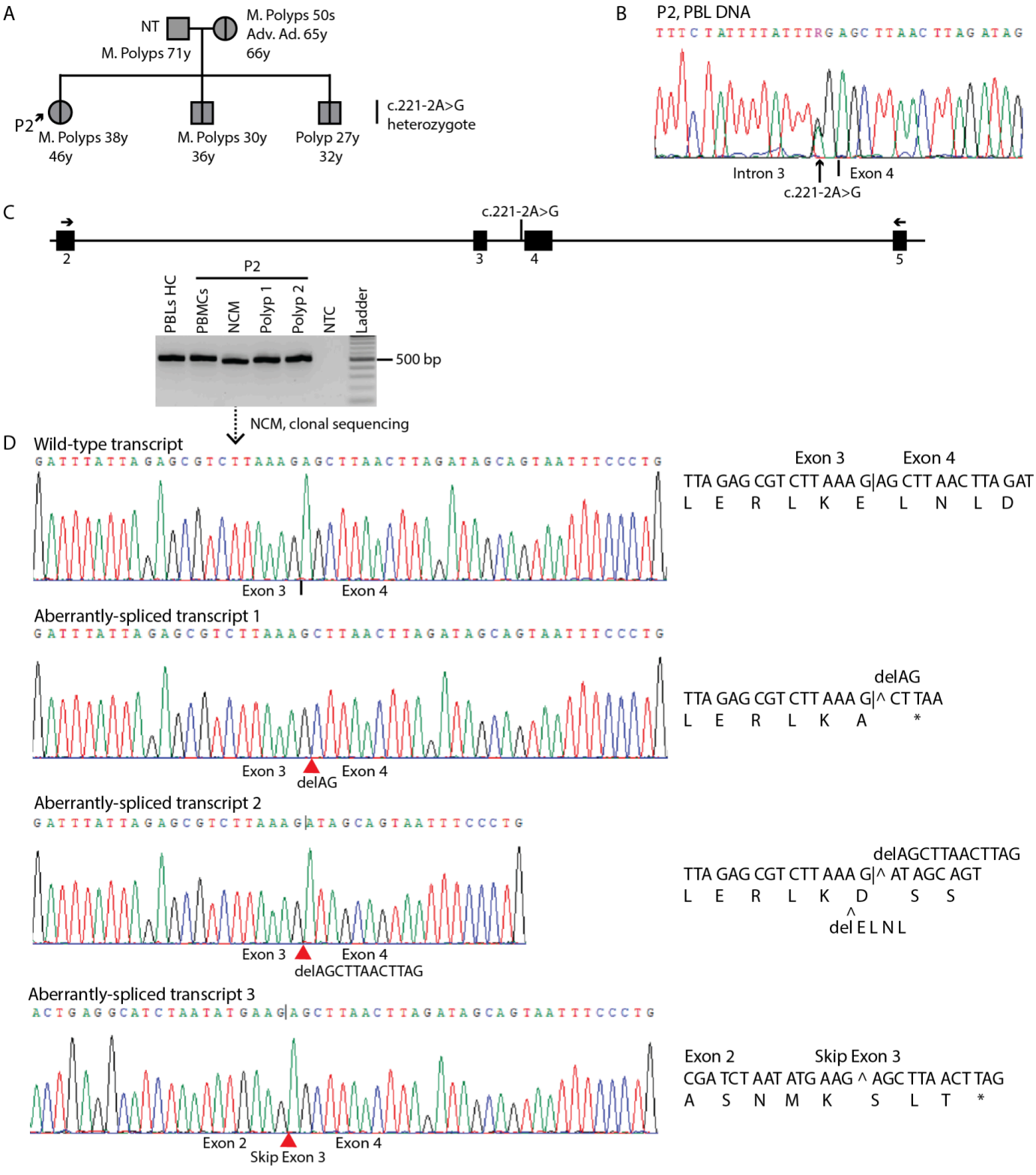



**Figure 1**

**Clinical pedigree and somatic mutational profile in Patient 1.**

**A:** Pedigree of Patient 1 (P1) harboring the *APC*c.1333C>T p.(Gln445Ter) pathogenic variant. The pedigree, assembled in consultation with P1 and P1's parents, revealed no reported family history of polyposis or colorectal cancer but identified cranial fasciitis (CF) in P1 and distant paternal relatives. Genetic testing was performed only in P1. The proband developed polyposis at age 31 years and rectal cancer at age 33 years.**B:** Somatic driver gene landscape in peripheral blood lymphocytes (PBL) and primary rectal tumor (PT) samples. Heatmap displays the number of unique HIGH- and MODERATE-impact somatic variants identified by whole exome sequencing in colorectal cancer-associated driver genes. Darker shades indicate a greater number of unique variants. The tumor exhibited a greater burden of loss-of-function alterations in *APC, POLE, ATM, MSH2,* and *MSH6* relative to PBL, and gain-of-function alterations in *NBAS,* consistent with increased molecular complexity and accumulation of driver-gene alterations within the tumor. Note: Somatic variants were retained if they passed Mutect2 filtering (FILTER = PASS), had a tumor sequencing depth ≥100× (normal ≥50×), a variant allele frequency (VAF) ≥5% in tumor samples, and a tumor log-odds (TLOD) score >10. Variants were annotated using the *Ensembl* Variant Effect Predictor (VEP), and only HIGH- and MODERATE-impact variants were included in downstream analyses. HIGH-impact variants included frameshift, stop-gained, and essential splice-site alterations predicted to severely disrupt protein function. MODERATE-impact variants primarily comprised missense and other protein-altering substitutions predicted to modify the amino acid sequence of the encoded protein without necessarily causing protein truncation or complete loss of

function. A *BRAF* alteration was identified in the PBL-derived variant set but was not retained in the filtered tumor call set, potentially reflecting differences in sequencing depth, allele frequency, filtering thresholds, or sample-specific clonal populations.




## Figure 2

**Pedigree and tissue-specific transcript effects of the *APC* c.221-2A>G splice variant in Patient 2.**

**A:** Parsimonious nuclear pedigree of P2. Grey shading indicates phenotype of multiple (M.) polyps and an advanced adenoma (Adv. Ad.) consistent with attenuated polyps, with earliest ages as diagnoses shown. Ages shown at the bottom are current ages. NT, not tested.

**B:** Sanger sequence electropherogram of P2 genomic DNA from peripheral blood leukocytes (PBL) showing heterozygosity for the *APC* c.221-2A>G pathogenic variant (PV), 2 bp upstream of exon 4.

**C:** Top, map of *APC* exons 2-5 and reverse-transcriptase (RT) PCR assay with respect to the location of the c.221-2A>G PV. Beneath, photo (cropped) of electrophoresis gel of column-purified RT-PCR products prior to cloning and sequencing of individual transcripts derived from two polyps, the normal colonic mucosa (NCM) and peripheral blood mononuclear cells (PBMCs) from P2 and peripheral blood lymphocytes (PBLs) from a colonoscopy-verified healthy control (HC), alongside a 100 bp ladder. The product sizes did not appear to differ significantly in P2 samples from the HC sample due to the small size differences observed in the most abundant aberrantly-spliced transcript (2 bp). A photo of the original gel is provided in Supplementary Figure 1.

**D:** Left, Illustrative electropherograms of the aberrantly spliced transcripts detected among P2 samples only, as shown for the NCM sample, with exon boundaries indicated by vertical lines and interpretations of errors (deletions) indicated by red triangles. Right, predicted impact of each altered splice transcript on translated amino acid sequences are shown with respect to the wild-type sequence above.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.xlsx
- SupplementaryTables.docx
- SupplementaryFigure1.tif