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# Expanding the Genetic Spectrum of Hereditary Polyposis Syndromes: A Multicenter Study from Argentina

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## Abstract

Hereditary polyposis syndromes (HPS) predispose to colorectal cancer, yet their genetic landscape in Argentina remains underrepresented. We analyzed 177 probands from 163 unrelated families recruited through three Argentine reference centers. Genetic testing included *APC* and *MUTYH* analysis by Sanger sequencing and MLPA, complemented by multigene panels and whole-exome sequencing (WES). Variants were classified following ACMG/AMP guidelines. Germline *APC* variants were identified in 75/177 probands (42.4%), pathogenic *MUTYH* variants in 11 (6.2%), and alterations in *BMPR1A* and *SMAD4*in 2 probands each. Within the *APC*-positive subset, 52 unique variants were detected, including seven novel truncating frameshift mutations and six large genomic rearrangements. Segregation analysis supported pathogenicity in available families. Genotype–phenotype correlations confirmed the enrichment of truncating variants within the mutation cluster region (MCR) in classic FAP, whereas distal variants predominated in attenuated forms. This first large-scale Argentine study characterizes *APC*- and *MUTYH*-related polyposis in the region. The identification of novel *APC* alleles and regionally recurrent *MUTYH* variants broadens the national reference framework, providing critical evidence to improve genetic diagnosis and clinical management in this specific population.

## INTRODUCTION

Hereditary polyposis syndromes (HPS) represent a clinically and genetically heterogeneous group of disorders characterized by the development of multiple adenomatous or hamartomatous polyps throughout the gastrointestinal tract, accompanied by an increased lifetime risk of colorectal cancer (CRC) and various extracolonic malignancies [1].

The two main inherited forms of adenomatous polyposis are APC-associated familial adenomatous polyposis (FAP) and MUTYH-associated polyposis (MAP). Germline pathogenic variants in *APC*, inherited in an autosomal dominant manner, cause FAP and account for most cases with a classic or attenuated adenomatous phenotype. In contrast, *MUTYH* variants lead to an autosomal recessive form of polyposis, typically characterized by a variable number of adenomas and a later onset of disease. Less frequent causes involve germline defects in *BMPR1A*, *SMAD4* (associated with Juvenile Polyposis Syndrome), *POLE*, *POLD1* (associated with Polymerase Proofreading-Associated Polyposis), and other genes, reflecting the increasing molecular complexity of polyposis-associated syndromes [2].

Although major advances have been made worldwide in the molecular diagnosis of HPS, comprehensive data from Argentina remain scarce. Most regional publications derive from single-center experiences or small series [3,4]; consequently, the genetic spectrum of HPS in Argentine patients is still only partially defined. This limited representation means many locally observed variants remain under-represented or absent from international repositories such as ClinVar, LOVD, and gnomAD. This limited representation makes accurate variant interpretation more difficult and may hide recurrent or population-specific alleles that are important for understanding disease variability [5].

In Argentina, molecular testing for polyposis has mainly relied on conventional methods—such as Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA)—while next-generation sequencing (NGS) approaches have only recently been incorporated in selected clinical and research settings. Although these technologies have significantly improved the diagnostic resolution of hereditary gastrointestinal cancer syndromes, their systematic adoption across institutions remains limited. Strengthening local diagnostic capabilities and variant-curation frameworks is therefore crucial to consolidate molecular evidence and improve precision medicine efforts for patients and families at risk.

Given the limited availability of multicenter genetic data, we conducted a collaborative study across three major Argentine reference centers—Hospital de Gastroenterología “Dr. Bonorino Udaondo,” Hospital El Cruce, and the Hereditary Cancer Program (PROCANHE) registry at Hospital Italiano de Buenos Aires. We analyzed a cohort of 177 probands with clinical suspicion of hereditary polyposis using MLPA and Sanger sequencing as core methodologies, complemented by available results from multigene panels and whole-exome sequencing (WES) in a subset of cases.

This study provides an integrative overview of the genetic landscape of hereditary polyposis syndromes in Argentina, with particular emphasis on *APC* and *MUTYH*, and additional findings in *BMPR1A*, *SMAD4*, and other polyposis-associated genes. By reporting several novel APC variants and curating multicenter-level data, our work contributes valuable evidence to enhance genetic diagnosis, clinical management, and variant interpretation in hereditary polyposis across Argentine patients.

## RESULTS

**Cohort Overview**

We analyzed 177 probands from 163 unrelated families across three Argentine reference centers (Supplementary Table S1). Of these, 173/177 (97.7%) fulfilled clinical criteria for classic/profuse adenomatous polyposis (145/173, 83.8%), attenuated polyposis (24/173, 13.9%), or mixed attenuated forms (4/173, 2.3%); the remaining 4/177 (2.3%) were classified as adenomatous polyposis of suspected hereditary origin. The cohort comprised 48.1% females and 51.9% males, with a median age at diagnosis of 35.8 years (range 10–78). Extracolonic features were documented in 55/144 (38.2%) evaluable probands, including gastric and duodenal polyps, desmoid tumors, osteomas, and thyroid lesions. Familial aggregation of polyposis and/or early-onset colorectal cancer was observed in 80.2% of probands.

**APC Variant Spectrum**

Across the entire cohort, 75/177 probands (42.4%) carried a germline *APC* variant, representing 52 unique alterations (Table 1 and Supplementary Table S2). The variant types included 24 frameshift (46.2%), 12 nonsense (23.1%), 9 missense (17.3%), 1 canonical splice-site (1.9%), and 6 large genomic rearrangements (LGRs) detected by MLPA (11.5%). Thirty-nine of 52 (75.0%) unique variants were previously reported in ClinVar, LOVD, or gnomAD. The remaining 13/52 (25.0%) were absent from all repositories; these included 7 novel truncating indels (detailed below) and the 6 LGRs, for which breakpoints were not mapped.

Figure 1 illustrates the distribution of APC variants along the protein, showing a characteristic clustering between codons 1200–1600, encompassing the β-catenin-binding and 20-amino-acid repeat domains. Several recurrent hotspots were identified, including c.3183_3187del (p.Gln1062_Ser1063delinsTer) in two probands and c.3927_3931delAAAGA (p.Glu1309AspfsTer4) in six. Fourteen unique variants (26.9%)—11 truncating (8 frameshift, 3 nonsense) and 3 missense—fell within the Mutation Cluster Region (MCR; codons 1282–1581).

Based on ACMG/AMP criteria (as curated via ClinVar and VarSome), the 39 previously reported variants were classified as 29 Pathogenic (P), 1 Likely Pathogenic (LP), 7 Variants of Uncertain Significance (VUS), 1 with conflicting interpretations, and 1 risk-factor allele. For the 6 LGDs, the five unique deletions were classified as Likely Pathogenic (LP) based on the PVS1 criterion, while the single large duplication was classified as a VUS.

**APC Large Genomic Rearrangements (LGRs)**

Six distinct LGRs were identified in 10 probands (Table 1 and S3). These included: ΔExons 09–10 (1 case); ΔPromoter B-A & 5'UTR (1); ΔPromoter B (3); ΔExons 10–16 partial (3); Duplication Exons 5–6 (1); and ΔExons 4–16 (1). Rearrangements involving the *APC* promoter region (Promoter B and Promoter B–A & 5'UTR) represent, to our knowledge, the first germline promoter deletions reported in Argentine polyposis cases. Figure 1 depicts the approximate genomic breakpoints, correlating the deleted segments with the APC protein domains. Most LGR carriers presented with profuse polyposis (>1000 adenomas), a positive family history, and variable extracolonic manifestations. Representative MLPA electropherograms are shown in Figure 2, and a detailed pedigree for the family carrying ΔExons 10–16 partial (case 171) is shown in Figure 3C, demonstrating vertical transmission and segregation consistent with dominant inheritance.

**Novel APC Variants**

We identified seven novel germline APC variants, all absent from ClinVar, LOVD, and gnomAD (accessed Oct 2025). All were frameshift variants predicted to generate premature stop codons and produce truncated, non-functional proteins. Three mapped within the MCR region (c.4098_4111del, c.4183dup, c.4359del). According to ACMG classification (VarSome Clinical), all seven were classified as Likely Pathogenic (PVS1 very strong + PM2 supporting), and have been subsequently submitted to ClinVar (Accession numbers: SCV007107120, SCV007107121, SCV007107123, SCV007107124, SCV007107125, SCV007107126, and SCV007107127).

Although these seven variants were not catalogued in ClinVar, truncating alterations affecting adjacent or overlapping residues have been reported previously, supporting a loss-of-function mechanism at these positions (Supplementary Table S2). Interrogation of somatic datasets (COSMIC v102 and cBioPortal, Oct 2025) revealed no exact matches, but multiple truncating events were observed within ±2 amino acids around codons 1367, 1395, and 1455, providing additional evidence for regional intolerance to truncation (Supplementary Table S3).

Representative Sanger electropherograms for five of the seven novel variants are shown in Figure 4A–E; DNA for Sanger confirmation was not available for two cases (010 and 088). Familial pedigrees for the c.4183dup variant carriers (cases 170 and 174) are shown in Figure 3A–B, corresponding to those with detailed family information. For the remaining novel APC variants, clinical and segregation data are summarized in Table 1.

**MUTYH variants**

Pathogenic or likely pathogenic *MUTYH* variants were detected in 11/177 probands (6.2%): eight biallelic (six homozygous, two compound heterozygous), consistent with *MUTYH*-associated polyposis, and three monoallelic carriers of risk alleles (Table 2 and Supplementary Table S2). Five unique *MUTYH* variants were identified; the c.1187G>A (p.Gly396Asp) accounted for 9/11 (82%) of cases, whereas c.536A>G (p.Tyr179Cys) was absent. Compared with *APC*-positive probands, MAP cases presented at older ages (mean 45.0 vs 29.7 years; Wilcoxon $p = 0.003$; Cliff's $\delta = 0.75$, very large effect).

**Variants in Other Polyposis-Associated Genes**

Pathogenic or likely pathogenic variants were also identified in *BMPR1A* (2/177; 1.1%) and *SMAD4* (2/177; 1.1%), showing overlapping phenotypes that combined AFAP-like and juvenile-type polyp burdens (Table 3). No pathogenic variants were detected in *POLE* or *POLD1* within the sequenced subset.

Beyond canonical polyposis genes, multigene panel and WES analyses identified additional variants in genes with emerging relevance to colorectal tumorigenesis (Table 3). One proband (046) carried compound alterations in *NEIL1* and *PMS2*, previously described by our group as a potential

interaction between base-excision and mismatch-repair (MMR) pathways [6]. Single heterozygous variants of uncertain significance were also identified in *MSH6* (p.Thr1008Ile), *MSH3* (p.Leu801Phe), and *BRCA1* (p.Arg7_Glu10delinsGln), each in one proband. These findings may contribute to atypical or overlapping polyposis phenotypes. Additional variants in genes with unconfirmed or low-evidence links to CRC (e.g., *ERCC2*, *ERCC4*, *FANCM*, *FLCN*, *PTCH1*) are listed in the Supplementary Table S2.

**Genotype–Phenotype Correlations**

Among *APC* carriers, truncating variants between codons 1200–1600 were predominantly associated with classic/profuse FAP, whereas distal truncations and missense variants were more common in attenuated (AFAP) forms. Median codon positions differed modestly (FAP = 1307 vs AFAP = 1113).

Cross-tabulation of FAP/AFAP phenotypes and MCR region showed no significant association (Fisher's p = 0.44; odds ratio ≈ 0.45). When restricted to unique variants, the pattern remained similar (p = 1.0). These data indicate that MCR-centered truncations predominate in classic FAP, whereas attenuated forms tend to harbor distal changes, consistent with previous genotype-phenotype studies [7]. Biallelic *MUTYH* carriers showed later onset and lower polyp burdens than classic FAP, while *SMAD4* and *BMPR1A* carriers displayed mixed adenomatous/juvenile phenotypes.

**Diagnostic Yield and Unresolved Cases**

In total, 83 of 177 probands (46.9%) remained genetically unresolved after comprehensive testing. This figure corresponds to the remaining cases once excluding 75 *APC*-positive probands, 11 *MUTYH* cases (biallelic and monoallelic), and 8 with pathogenic, likely pathogenic, or VUS variants in other genes (*BMPR1A*, *SMAD4*, *MSH6*, *MSH3*, *BRCA1*, and *PMS2*). Among the 18 probands analyzed by WES, 9 (50%) lacked a clearly causal genotype in currently recognized predisposition genes. These findings underscore the persistent diagnostic gap in Argentine polyposis cohorts and highlight the need for broader molecular approaches and systematic variant re-evaluation.

## DISCUSSION

This multicenter study provides a comprehensive overview of the germline genetic landscape underlying clinically defined adenomatous polyposis in Argentina. By integrating data from three national referral centers, we identified *APC* variants as the predominant cause of disease, followed by *MUTYH* alterations and a smaller subset of variants in other DNA-repair or polyposis-associated genes. The overall diagnostic yield (~ 55%) parallels that reported in other national cohorts [8,9], confirming that despite methodological differences, most clinically defined polyposis cases are explained by *APC* or *MUTYH* defects.

Consistent with previous population-based analyses [9–12], the APC variant spectrum in our cohort was dominated by truncating alterations distributed across canonical hotspots between codons 1200–1600, encompassing the β-catenin and 20-amino-acid repeat domains. Comparable clustering has been described in European, Asian, and North American cohorts, reinforcing the universal nature of the APC mutation cluster region (MCR)[13–15]. In contrast, truncations located at the N-terminal or distal 3' ends of *APC* were predominantly associated with attenuated forms, in line with the positional genotype–phenotype trend repeatedly observed across independent FAP registries [16–18]. Our data replicates this positional trend, as FAP probands showed MCR-centered variants, whereas AFAP cases carried alterations outside this interval.

Large genomic rearrangements (LGRs) accounted for approximately 13% of APC-positive cases in our cohort, a frequency comparable to that observed in other contemporary FAP series, where LGRs constitute ~ 5–14% of pathogenic APC variants [19,20]. Notably, four of the rearrangements identified here involved the APC promoter region (Promoter B and Promoter B–A & 5'UTR). To our knowledge, these represent the first germline promoter deletions reported in Argentine polyposis cases. Although promoter deletions in *APC* are uncommon, they have been recognized as pathogenic mechanisms leading to reduced transcription of the APC-1B isoform and contributing to classic FAP phenotypes [21–23]. Their detection in multiple unrelated Argentine families expands the mutational spectrum of APC and underscores the need to include upstream regulatory regions in local diagnostic pipelines. Given that MLPA and CNV analyses are not yet uniformly implemented across Argentina, the incorporation of copy-number testing into standardized workflows is essential to minimize false negatives, following international and regional recommendations [24].

Seven APC variants—accounting for 13.5% of the 52 unique *APC* alterations identified—were novel, being absent from ClinVar, LOVD, and gnomAD. All were truncating events predicted to introduce premature stop codons and result in loss of function. Three of these (c.4098_4111del, c.4183dup, and c.4359del) mapped within the mutation cluster region (MCR), a functionally constrained interval enriched for truncating pathogenic events, while the remaining four occurred in domains essential for β-catenin regulation. Similar proportions of novel truncating *APC* variants have been reported in other FAP cohorts from Europe and Asia, confirming that newly identified alleles continue to emerge as sequencing expands across populations [25,26]. Adjacent or overlapping truncations at equivalent residues have consistently been documented as pathogenic in major FAP registries [27], reinforcing a shared loss-of-function mechanism through nonsense-mediated decay.

Segregation analyses available for several families demonstrated co-segregation of these variants with the FAP phenotype across independent branches, strengthening causal inference. According to ACMG/AMP recommendations and the ClinGen Hereditary Cancer Variant Curation Expert Panel guidelines [28–30], these truncating events fulfill very strong (PVS1) and supporting (PM2) criteria, warranting classification as *Likely Pathogenic*

(Class 4). Taken together, these findings consolidate the pathogenic relevance of the newly identified *APC* alleles and broaden the mutational spectrum in Argentina, revealing locally recurrent variants that may represent regional founder events or independent mutational hotspots requiring validation in larger cohorts.

*MUTYH*-related polyposis accounted for 6.2% of all probands, a frequency consistent with other Western cohorts [31–33]. The founder *p.Gly396Asp* variant predominated (82%), whereas *p.Tyr179Cys*—common in Northern Europe—was absent, suggesting regional allele heterogeneity, as previously proposed for South American populations [34,35]. Clinically, MAP cases presented later and with fewer adenomas than *APC*-positive probands, consistent with the attenuated course widely described in MAP [36]. Interestingly, monoallelic *MUTYH* variants were found in two cases, raising the possibility of modest CRC risk among heterozygous carriers, as supported by recent analyses [34,37].

The heterogeneity of genetic diagnosis in this cohort is exemplified by Case 046. This patient, reported in detail previously by our group [6], highlights the potential for the coexistence of defects in distinct DNA-repair pathways. Initially, a heterozygous *NEIL1* variant (c.506G > A), affecting a BER glycosylase, was considered a plausible candidate for the polyposis phenotype. However, subsequent tumor profiling revealed an MMR-deficient mutational signature and high tumor mutational burden (TMB), prompting extended germline re-analysis. This re-evaluation uncovered a concomitant large deletion in *PMS2* (exons 2–11), confirming dual pathogenic alterations in both BER and MMR pathways. This finding illustrates how cross-pathway defects can explain atypical phenotypes and underscores the importance of a combined tumor-germline diagnostic approach, particularly in the interpretation of isolated BER variants in hereditary polyposis contexts.

In addition to the primary *APC* and *MUTYH* variant spectrum, pathogenic alterations were also identified in *BMPR1A* and *SMAD4*, each accounting for approximately 1% of the proband cohort. Clinically, these cases presented with overlapping adenomatous and juvenile phenotypes. This phenotypic ambiguity is well-documented; while *BMPR1A* and *SMAD4* are the canonical genes for Juvenile Polyposis Syndrome (JPS), the associated variability in polyp histology and number frequently overlaps with attenuated adenomatous presentations [38,39]. The specific *SMAD4* nonsense variants found in our cohort, p.Gln248Ter and p.Trp302Ter, are established pathogenic alleles [40]. Their presentation in this context, rather than as classic JPS, supports a broader functional spectrum for *SMAD4*-mediated predisposition. These results reinforce the recommendation that *BMPR1A* and *SMAD4* analysis should be considered in the diagnostic workup for patients presenting with hamartomatous or mixed polyp histology.

Beyond the canonical polyposis genes, variants were also identified in a broader set of DNA-repair and tumor-suppressor genes, including *MSH3*, *MSH6*, *BRCA1*, *ERCC2*, *ERCC4*, *FANCM*, *FLCN*, and *PTCH1*. The *MSH3* missense variant p.Leu801Phe illustrates the significant challenge of interpreting VUS in emerging polyposis-associated genes. Biallelic pathogenic variants in *MSH3* are now recognized as a rare cause of *MSH3*-associated adenomatous polyposis [41,42]. Our proband, however, carried only this single monoallelic VUS. The clinical significance of monoallelic *MSH3* variants remains unresolved, though the possibility of a second, undetected germline hit (such as a large rearrangement or deep intronic mutation) cannot be excluded, a scenario emphasized in other unresolved *MSH3* cases [43].

Similarly, the *MSH6* variant p.Thr1008Ile is a VUS of high interest. This specific variant has been functionally demonstrated to reduce MMR activity *in vitro* [44] and may contribute to attenuated adenomatous phenotypes that do not meet the criteria for classic Lynch syndrome [45]. These findings reinforce the concept of an expanding spectrum of MMR-related phenotypes that can overlap with polyposis presentations.

Finally, VUS were identified in several other genes involved in genomic maintenance (*BRCA1*, *ERCC2*, *ERCC4*, *FANCM*) and cell signaling (*FLCN*, *PTCH1*). While heterozygous pathogenic variants in genes like *FANCM* have been variably associated with a general increased cancer risk [46,47], robust evidence linking them directly to a colorectal predisposition or polyposis phenotype is still limited. Consequently, these variants were classified as contextual findings, and their clinical significance in this setting requires further validation in larger case-control studies.

Despite a comprehensive diagnostic workflow—which included targeted *APC*/*MUTYH* screening (MLPA/Sanger) complemented by extended WES/NGS panel analysis—approximately 45% of our proband cohort remained genetically unresolved. This diagnostic gap is consistent with previous multicenter studies, which frequently report that 30–50% of polyposis cases remain genetically unexplained [48]. These undiagnosed cases likely reflect the inherent limitations of current standard assays. The underlying etiologies may include somatic mosaicism, deep-intronic -variants (such as those creating pseudoexons), promoter variants missed by sequencing, or complex structural variants beyond the resolution of MLPA [48–50]. To close this gap, expanding analyses to include, for example, -long-read sequencing and -RNA-based assays. Furthermore, at a national level, strengthening inter-institutional networks and developing unified variant databases will be critical for improving molecular diagnosis and refining surveillance strategies for hereditary CRC syndromes across our population.

In summary, this study represents the first large-scale, multicenter characterization of *APC*- and *MUTYH*-related polyposis in Argentina. The identification of novel truncating *APC* variants, recurrent *MUTYH* alleles, and complementary alterations in DNA repair genes broadens our understanding of hereditary colorectal cancer predisposition within the country. Continued efforts through coordinated national and regional collaborations will be essential to refine variant interpretation, uncover population-specific patterns, and resolve the remaining diagnostic gap in hereditary colorectal cases. Replicating similar initiatives across other Latin American cohorts will ultimately help define regional mutational backgrounds and improve access to precision diagnostics throughout the region.

# METHODS

## Study Design and Patient Cohorts

This multicenter, collaborative study included 177 probands from 163 unrelated families. The clinical diagnosis of hereditary polyposis was established based on a combination of criteria, including polyp number and histology, age at onset, and a family history of polyposis and/or CRC. Based on these criteria, 173/177 (97.7%) probands were sub-classified as having attenuated or classic/profuse adenomatous polyposis, while the remaining 4/177 (2.3%) were classified as adenomatous polyposis of undefined hereditary origin (Supplementary Table S1). Patients were recruited through local hereditary cancer registries from three Argentine reference centers: the Hereditary Cancer Registry (PROCANHE) at Hospital Italiano de Buenos Aires (HIBA, CABA), Hospital de Gastroenterología “Dr. Bonorino Udaondo” (HGBU, CABA), and Hospital El Cruce (HEC, Florencio Varela, Buenos Aires). The study integrated newly generated genetic data with curated information from institutional records.

The study protocol was approved by the Institutional Review Boards (IRB) of all participating centers. All participants provided written informed consent during genetic counseling, in accordance with institutional, national and international ethical standards.

**Germline Variant Analysis.**

**HGBU and HEC Cohorts.** A two-phase workflow was implemented. **Phase I (MLPA and Sanger sequencing)**: Genomic DNA was extracted from peripheral blood as described previously [51]. Multiplex ligation-dependent probe amplification (MLPA) was performed using the SALSA MLPA P043 *APC* probe mix (MRC-Holland) to detect large genomic rearrangements (LGRs) in *APC* and point variant alterations in *MUTYH*. Negative samples for LGRs were subsequently analyzed by Sanger sequencing, covering coding exons 2 to 16 of *APC* and ± 5 bp of flanking intronic regions (primer sequences are detailed in Supplementary Table 4). **Phase II (WES Subset)**: A total of 18 probands (15 from Udaondo and 3 from HIBA) underwent whole-exome sequencing (WES) as part of a previously published study by our group [6]. Twelve cases were included in that publication, while six are reported here for the first time. Primary data, including WES (QC, primary and secondary steps) and MLPA fragment separation, was performed at the Institute for Molecular Medicine Finland (FIMM, University of Helsinki). Subsequent downstream analysis and interpretation were conducted by the study team using VarSeq® software (Golden Helix) for WES data and GeneMapper 5.0 (Applied Biosystems) for MLPA profiles. *APC* gene analysis by Sanger sequencing was carried out at the HIBA Sequencing Facility.

**HIBA Cohort.** Genetic data for the HIBA cohort was obtained from the PROCANHE registry. This test included multigene hereditary cancer panels (covering *APC*, *MUTYH*, *BMPR1A*, *SMAD4*, and other relevant genes) and MLPA assays performed by the institutional sequencing service. For three probands, WES data generated at FIMM were integrated into this analysis. All HIBA results were reviewed and reclassified by the study team to ensure consistency with ACMG/AMP criteria and harmonization of variant nomenclature across cohorts.

## Variant Annotation and Classification

The recurrence or novelty of each variant was determined through searches in ClinVar, LOVD, and gnomAD, complemented by manual review. In addition, somatic mutation databases were interrogated, including the Catalogue of Somatic Mutations in Cancer (COSMIC, v102; March 2025 release) and the cBioPortal for Cancer Genomics (accessed October 2025). Findings were used to document somatic recurrence or positional clustering.

Novel variants were interpreted according to the ACMG/AMP 2015 guidelines[28] using VarSome Clinical (ACMG computational scoring). Variant nomenclature followed the Human Genome Variation Society (HGVS) guidelines [52]. Reference transcripts were as follows: *APC* (NM_000038.6), *MUTYH* (NM_001048174.2), *BMPR1A* (NM_004329.3), *SMAD4* (NM_005359.6), *MSH3* (NM_002439.5), *MSH6* (NM_000179.3), and *BRCA1* (NM_007294.4). Reference sequences for additional genes are listed in Supplementary Table S2. Variants absent from all major databases were classified as novels. Large genomic deletions (LGDs) were reported as unique events. Deletions affecting one or more exons were classified as Likely Pathogenic (LP), as they are considered null variants meeting the PVS1 criterion[28], a classification supported by the recently published *APC*-specific ClinGen framework[30]. Large duplications were categorized as a VUS due to its more complex functional interpretation, as precise breakpoints were not determined.

Variant extraction and database queries were performed using custom scripts developed in R (v4.4.1). These scripts were used to filter, annotate, and extract relevant data subsets from the local database. All database searches were last updated in October 2025 (gnomAD v4.1, ClinVar June 2025, LOVD May 2025, COSMIC v102).

## Data Summary and Visualization

Unique variants were counted per gene and classified by molecular type. Frequency was expressed as both the number of unique variants and the number of affected probands. *APC* lollipop plot was generated using R (v4.4.1) ggplot2 package.

## Statistical Analysis

Comparative analyses were performed using non-parametric statistical tests. Differences in age at diagnosis between *APC* and *MUTYH* carriers were assessed with the Wilcoxon rank-sum test, with Cliff's delta calculated for effect size. Associations between categorical variables—such as phenotype (FAP vs. AFAP) and variant localization relative to the APC Mutation Cluster Region (MCR)—were evaluated using Fisher's exact test. All analyses used a two-tailed significance threshold of $p < 0.05$.

## Declarations

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**Author contributions**

Conceptualization, JNS, PP, and WHP. Data curation, FIMM. Formal analysis, JNS, APO, ACM, PP, and WHP. Funding acquisition, APO, CAV, PP, and WHP. Investigation, JNS, APO, ACM, PP, and WHP. Methodology, JNS, APO, ACM, HG-R, TTN, PP, and WHP. Project administration, PP and WHP. Resources, MC, AKC, TAP, PGK, MLG, KY, JG, CAV, PP, and WHP. Software, JNS, APO and WHP. Supervision, TTN, PP, and WHP. Validation, JNS, APO, PP, and WHP. Visualization, JNS, PP, and WHP. Writing-original draft, JNS and WHP. Writing-review and editing, JNS, APO, ACM, TTN, PP, and WHP. All authors contributed to the article and approved the submitted version.

**Data availability statement**

The novel variants identified in this study have been submitted to the ClinVar database under submission numbers SUB15798962, SUB15798952, SUB15798943, SUB15798936, SUB15798930, SUB15798905, and SUB15797812; and Accession numbers: SCV007107127, SCV007107126, SCV007107125, SCV007107124, SCV007107123, SCV007107121, and SCV007107120, respectively. The Whole Exome Sequencing (WES) datasets generated and/or analyzed during the current study are not publicly available due to patient privacy protections from our IRB approvals, but are available from the corresponding author on reasonable request. However, all variants fulfilling the selection criteria are fully listed in the Supplementary Tables. Genetic and phenotype data of the entire cohort of patients are provided within the manuscript or supplementary information files.

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**ADDITIONAL INFORMATION**

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethics statement**

The studies involving human participants were reviewed and approved by the Institutional Review Boards from the Hospital Italiano de Buenos Aires (CABA), Hospital de Gastroenterología "Dr. Bonorino Udaondo" (CABA), and Hospital El Cruce (Florencio Varela, Buenos Aires). The patients/participants provided their written informed consent to participate in this study.

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## Tables

**TABLE 1 Novel truncating and large-scale APC germline variants and corresponding clinical phenotypes identified in Argentine FAP families.**

| Variant type | Patient ID | Sex | cDNA (NM_000038.6) | Protein (NP_000029.2) | Age FAP/CRC (yrs) [1] | Extracolonic features (Age) [1] | Family history FAP/CRC | Familial cases (n) [2] | Carriers per family | ACMG Class [3] |
|---|---|---|---|---|---|---|---|---|---|---|
| A. indels | 023 | F | c.4183dup | p.Ser1395Lysfs*3 | 35 | gastric polyps, abdominal desmoid (35, 36) | Yes/Yes | na | 1 | LP |
| | 170 | F | | | 18 | duodenal ad. (18) | Yes/Yes | FAP (6), CRC (4) | 3 | |
| | 174 | F | | | 12 | duodenal ad. / fundic gland polyps (13) | Yes/Yes | FAP (8), EC (1), PC (1) | 3 | |
| | 033 | F | c.4359del | p.Asn1455Ilefs*18 | 14 | | Yes/Yes | CRC (1) | 2 | LP |
| | 005 | M | c.4098_4111del | p.Gln1367Serfs*3 | 24/24 | | No/No | | 1 | LP |
| | 010 | F | c.1271dup | p.Glu425Glyfs*4 | 29/29 | | No/No | | 1 | LP |
| | 014 | M | c.2450del | p.Gly817Alafs*3 | 17 | | No/No | | 1 | LP |
| | 075 | M | c.2952dup | p.Ser985Ilefs*4 | 19/21 | | Yes/Yes | na | 1 | LP |
| | 088 | F | c.2520dup | p.Asp842Argfs*2 | 24 | | Yes/Yes | na | 1 | LP |
| | | | | | | | | | | |
| B. LGRs | 053 | F | ΔPromoter B | | 33/33 | | No/Yes | CRC (1) | 1 | LP |
| | 179 | F | | | 23/23 | duodenal papilla adenocarcinoma / fundic gland polyps (32) | Yes/Yes | na | 1 | |
| | 183 | M | | | 12 | | Yes/Yes | na | 1 | |
| | 060 | F | ΔExons 10-16(partial) | | 45/47 | gastric polyps (45) | Yes/Yes | CRC (1) | 1 | LP |
| | 171 | M | | | 23 | duodenal ad. (30) | Yes/Yes | FAP (6), CRC (3), TC (1), DT (1) | 1 | |
| | 173 | M | | | 15 | duodenal ad. / fundic gland polyps (26) | Yes/Yes | na | 1 | |
| | 012 | M | ΔPromoter B - A and 5UTR | | 15 | | Yes/Yes | CRC (1) | 1 | LP |
| | 004 | M | ΔExons 11-12 | | 33/33 | desmoid tumor / duodenal ad. (33) | Yes/Yes | Uterus (1) | 1 | LP |
| | 099 | F | Duplication Exons 5-6 | | 29 | | Yes/Yes | na | 1 | VUS |
| | 104 | M | ΔExons 4-16 | | 30 | | Yes/Yes | na | 1 | LP |

FAP: Familial Adenomatous Polyposis (severe condition, >1000 polyps); CRC: Colorectal Cancer; EC: Endometrial Cancer; PC: Pancreatic Cancer; TC: Thyroid Cancer; DT: Desmoid Tumor; LP: Likely Pathogenic; VUS: Variant of Uncertain Significance; na: not available; M: Male; F: Female

1 Age at diagnosis is shown; 2 Segregation data are summarized for index families, full pedigrees are available in Supplementary Table S1; 3 ACMG classification for novel truncating variants per VarSome Clinical (Sept 2025), LGRs classified as VUS as no breakpoints were defined.

**TABLE 2 MUTYH germline variants identified in Argentine polyposis families.**

| cDNA NM_001048174.2 | Protein NP_001041639.1 | Type | Carriers hom/het | ClinVar ID | ClinVar Significance[1] | gnomAD genome/exome | Affected cases |
|---|---|---|---|---|---|---|---|
| c.1103G>A | p.Gly368Asp | ms | 6/3 | 5294 | P/LP | 0.00332804/nf | 9 |
| c.205C>T | p.Arg69* | ns | 0/1 | 140827 | P | 3.29x10-5/nf | 1 |
| c.1143_1144dup | p.Glu382Glyfs*43 | fs | 0/1 | 127831 | P/LP | 0.000262812/nf | 1 |
| c.1063del | p.Ala357Profs*23 | fs | 0/1 | 134860 | P/LP | nf/nf | 1 |
| c.637C>T | p.Arg213Trp | ms | 0/1 | 185274 | P/LP | 1.97x10-5/nf | 1 |

ms: missense; ns: nonsense; fs: frameshift; nf: not found; hom: homozygous; het: heterozygote; P: pathogenic; LP: likely pathogenic; 1 ClinVar classification as of Sept 2025.

**TABLE 3 Non-APC/MUTYH germline variants detected by WES or Multigene Panel in Argentine polyposis families.**

| Gene | cDNA[1] | Protein[1] | Patient ID | Sex | Age at diagnosis (yrs)[2] | Polyp type (count) | Extracolonic features (Age)[2] | Family history FAP/CRC | ClinVar Classification[3] |
|---|---|---|---|---|---|---|---|---|---|
| BMPR1A | c.1394del | p.Pro465Argfs*33 | 019 | M | FAP (25), CRC (39) | ad. (>1000) | No | No/No | P/LP |
| | ΔExons 6-10 | | 109 | F | FAP (48), CRC (48) | ad. (>1000) | No | na/na | |
| SMAD4 | c.742C>T | p.Gln248* | 006 | F | AFAP (60) | ad. (20) | gastric polyps (60) | No/No | P |
| | c.905G>A | p.Trp302* | 027 | M | JPS (18) | Juvenile (<100) | No | No/Yes | P |
| MSH3 | c.2401C>T | p.Leu801Phe | 110 | F | FAP (42) | ad. (>1000) | No | Yes/Yes | VUS |
| MSH6 | c.3023C>T | p.Thr1008Ile | 048 | M | FAP (22) | ad. (>1000) | lipoma, osteoma (24) | Yes/No | LP/VUS |
| PMS2 | ΔExons 2-11 | | 046 | M | Mixed Polyposis (60), CRC (60) | mixed serrated ad. | No | No/Yes | |
| NEIL1 | c.506G>A | p.Gly169Asp | 046 | | | | | | na |
| BRCA1 | c.20_28del | p.Arg7_Glu10delinsGln | 001 | M | FAP (42) | ad. (>1000) | No | No/No | VUS |

FAP/AFAP: Familial or Attenuated Adenomatous Polyposis; JPS: Juvenile Polyposis Syndrome; CRC: Colorectal Cancer; ad.: adenomas; na: not available; P: Pathogenic; LP: Likely Pathogenic; M: Male; F: Female.

1 HGVSc. and HGVSp., notations correspond to reference transcripts described in Methods; 2 Age at diagnosis is shown; 3 ClinVar classification as of Sept 2025; All variants were heterozygous, PMS2 + NEIL1 compound findings in same proband.

## Figures

FIGURE 1

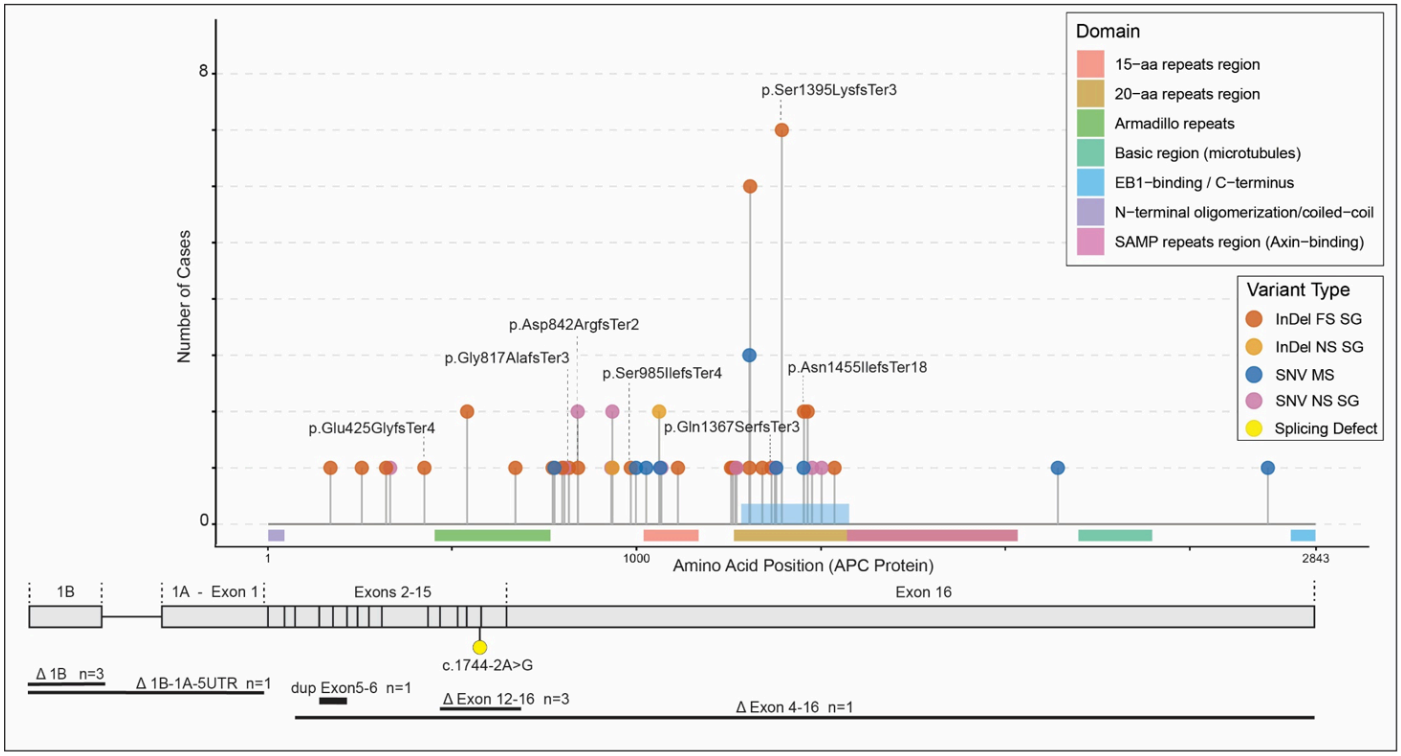



**Figure 1**

**Distribution of germline APC variants identified in Argentine familial adenomatous polyposis probands.**

Lollipop diagram illustrates the position and type of *APC* variants detected in the study cohort (n = 75 probands, 52 unique variants). Circles above the schematic protein structure indicate point alterations (SNVs and small InDels), whereas bars below represent large genomic rearrangements (LGRs). Colors denote the variant´s specific mechanism and its protein-level consequence, categorized as: SNV Missense (SNV MS), SNV-mediated Stop Gain (NS SG; nonsense), and InDel-mediated Stop Gain (FS SG; Frameshift). The splicing defect variant is indicated separately on the genomic track. Domain annotation follows the reference protein NP_000029.2, highlighting the armadillo repeat, β-catenin binding region, and 20-amino-acid repeat clusters. The mutation cluster region (MCR; codons 1282–1581) is shaded (clear blue). Novel truncating variants are labeled by protein change (e.g., p.Gln1367SerfsTer3, p.Asn1455IlefsTer18). Large deletions and duplications (e.g., ΔPromoter 1B–1A–5'UTR, ΔExons 12–16, DupExons5–6) are shown below the structure with the corresponding number of probands (n).

FIGURE 2

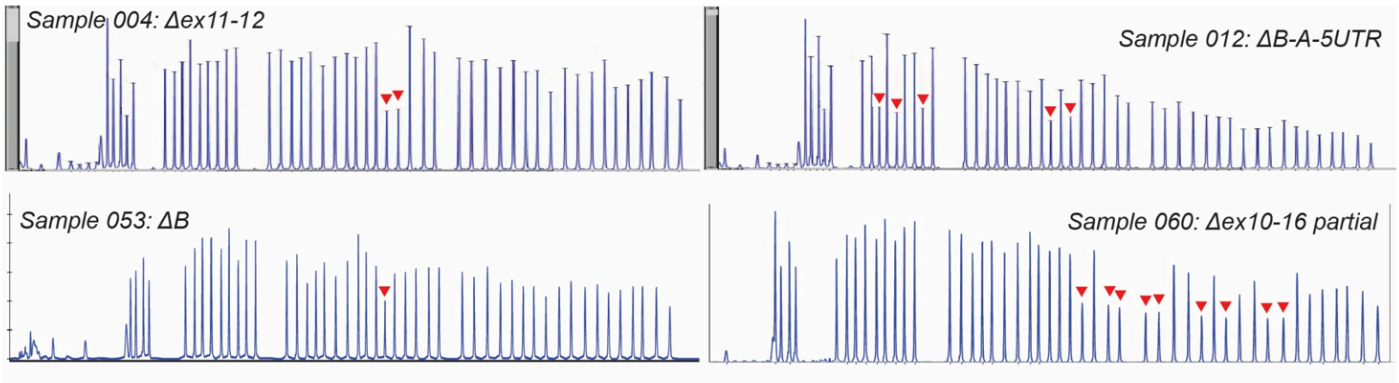



**Figure 2**

**Representative MLPA profiles of APC large genomic rearrangements (LGRs) identified in Argentine probands.**

Electropherograms show relative probe signal intensities across APC exons, obtained using the SALSA MLPA P043 APC kit (MRC-Holland). Arrowheads indicate reduced emission peaks at deleted exons. The average probe ratios for each sample are indicative of a heterozygous deletion: Sample 004 [ΔEx11–12] (0.54 ± 0.07); Sample 012 [ΔB–A–5'UTR] (0.55 ± 0.05); Sample 053 [ΔB] (0.52 ± 0.07); and Sample 060 [ΔEx10–11 partial] (0.51 ± 0.07).

**FIGURE 3**

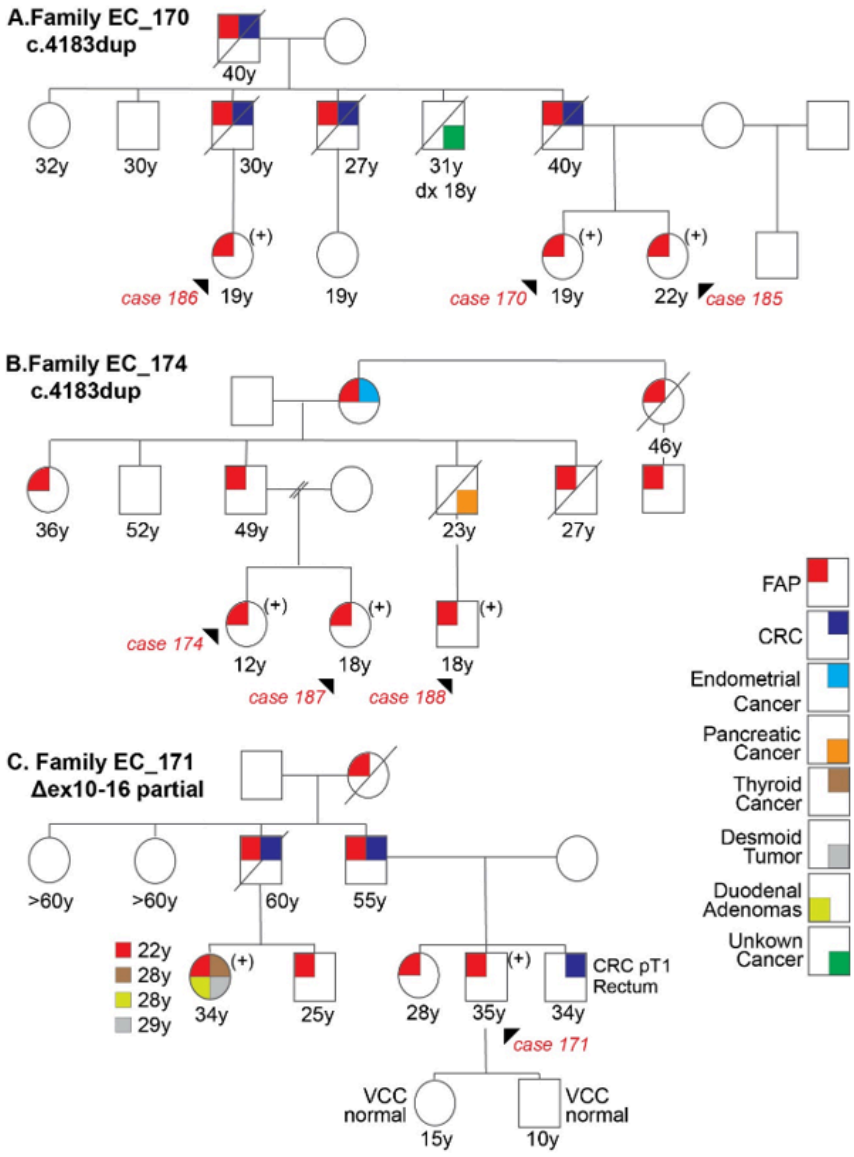



**Figure 3**

**Pedigrees of Argentine families carrying novel APC variants and large genomic rearrangements.**

(A) Family EC_170 and (B) Family EC_174, both harboring the APC c.4183dup variant; and (C) Family EC_171 carrying the deletion ΔEx10–16partial. Affected individuals are shown with filled symbols indicating the corresponding tumor or phenotype, as detailed in the color legend. Black arrowheads denote the probands included in this study, and red labels indicate internal case IDs. (+) marks confirmed heterozygous carriers of the variant under study. Ages at diagnosis (y = years) are indicated below each symbol. These pedigrees illustrate variant segregation among affected relatives, consistent with autosomal dominant inheritance of APC-associated polyposis.

FIGURE 4

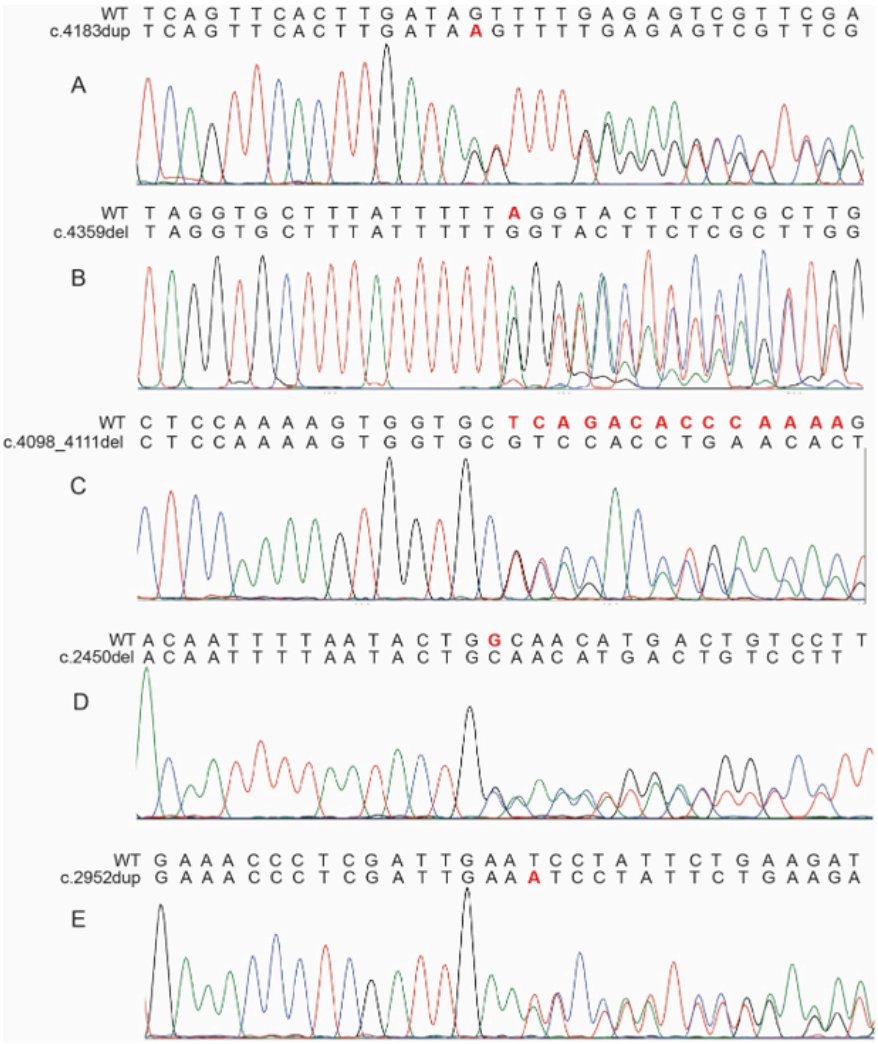



**Figure 4**

**Sanger sequencing chromatograms confirming five novel APC variants identified in Argentine probands.**

Electropherograms show representative reads compared with the wild-type (WT) reference sequence. Each panel corresponds to a distinct proband carrying a novel truncating variant: (A) Case 023, c.4183dup; (B) Case 033, c.4359del; (C) Case 005, c.4098_4111del; (D) Case 014, c.2450del; and (E) Case 075, c.2952dup.
Altered nucleotides are highlighted in red, and the overlapping traces illustrate the frameshift pattern typical of insertion or deletion events. Variants were confirmed by bidirectional sequencing and classified as *likely pathogenic* according to ACMG/AMP criteria.

## Supplementary Files

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

- SupplementaryTablesS1S4.pdf