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ARTICLE

Gene-specific ACMG/AMP classification criteria for germline *APC* variants: Recommendations from the ClinGen InSiGHT Hereditary Colorectal Cancer/ Polyposis Variant Curation Expert Panel

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ABSTRACT

Purpose: The Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel (VCEP) was established by the International Society for Gastrointestinal Hereditary Tumours and the Clinical Genome Resource, who set out to develop recommendations for the interpretation of germline *APC* variants underlying Familial Adenomatous Polyposis, the most frequent hereditary polyposis syndrome.
Methods: Through a rigorous process of database analysis, literature review, and expert elicitation, the *APC* VCEP derived gene-specific modifications to the ACMG/AMP (American College of Medical Genetics and Genomics and Association for Molecular Pathology) variant classification guidelines and validated such criteria through the pilot classification of 58 variants.
Results: The *APC*-specific criteria represented gene- and disease-informed specifications, including a quantitative approach to allele frequency thresholds, a stepwise decision tool for truncating variants, and semiquantitative evaluations of experimental and clinical data. Using the *APC*-specific criteria, 47% (27/58) of pilot variants were reclassified including 14 previous variants of uncertain significance (VUS).
Conclusion: The *APC*-specific ACMG/AMP criteria preserved the classification of well-characterized variants on ClinVar while substantially reducing the number of VUS by 56%

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(14/25). Moving forward, the *APC* VCEP will continue to interpret prioritized lists of VUS, the results of which will represent the most authoritative variant classification for widespread clinical use.

Introduction

Heterozygous germline pathogenic variants in the tumor suppressor gene *APC* (*adenomatous polyposis coli*, HGNC:583) lead to classic or attenuated familial adenomatous polyposis (FAP, MONDO: 0021057), an autosomal dominant condition characterized by the growth of hundreds and thousands of colorectal adenomatous polyps, which almost invariably progresses to early-onset colorectal cancer if left untreated.[1,2] As a result, clinical management guidelines for endoscopic surveillance and risk-reducing surgery are in place.[3-5] Pathogenic germline *APC* variants demonstrate variable expressivity manifested in different age of onset, polyp burden, and the presence of extra-colonic features, which include duodenal adenoma, duodenal carcinoma, and gastric carcinoma with an increasing incidence noted in recent years.[6] Other extra-intestinal manifestations include osteomas, desmoids, epidermoid cysts, congenital hypertrophy of the retinal pigment epithelium, adrenal adenomas, hepatoblastomas, medulloblastomas, and papillary thyroid carcinomas.[7]

Historically, the term attenuated FAP (AFAP) (MONDO: 0016362) was used to distinguish a milder form of the disease from classic FAP (MONDO: 0021055). However, it has been increasingly recognized that the dichotomy between classic and AFAP is somewhat arbitrary and does not fully capture the continuous spectrum of the colorectal phenotype and the complexity of extra-colonic lesions. Hence, AFAP is often regarded as a legacy description and is no longer recommended, as are other historical nomenclatures for specific phenotypes such as Gardner or Turcot syndromes. As a result, the terms classic and AFAP are combined and treated as 1 entity (MONDO: 0021057) when discussing the pathogenicity of *APC* variants in relevant phenotypes.

During the last 3 decades, thousands of rare or private pathogenic *APC* germline variants have been identified in FAP families. In parallel, advances in high-throughput sequencing, expansion in large hereditary cancer gene panels, and genome-scale screening in individuals with unrelated phenotypes or healthy controls have led to the detection of rare *APC* variants at a rate several orders of magnitude higher than in targeted sequencing, adding to the challenge of variant pathogenicity interpretation. The lack of existing data, information sharing, and consensus on variant classification have rendered most of these findings as either variants of uncertain clinical significance (VUS) or variants with conflicting interpretation. ClinVar currently lists 10,212 *APC* germline variants, 66% of which are VUS and only 8% overlap with the *APC* locus-specific database (LSDB) (retrieved 04/05/2022).

To address this issue, expert bodies are curating actively under the governance of Clinical Genomic Resource (ClinGen)—a National Institute of Health-funded effort dedicated to building a central resource that defines the clinical relevance of genes and variants.[8] For well-defined genes and diseases, ClinGen variant curation expert panels (VCEP) submit variant classification with their accompanying evidence to ClinVar and the ClinGen Evidence Repository—the first regulatory-grade human variant database. The works of other VCEPs are summarized

Abbreviations

ACMG – American College of Medical Genetics and Genomics
AFAP – attenuated FAP
AMP – Association for Molecular Pathology
APC – *adenomatous polyposis coli*
B – benign
BA – benign stand alone
BP – benign supporting
BS – benign strong
ClinGen – Clinical Genome Resource
EMBL-EBI – European Molecular Biology Laboratory and European Bioinformatics Institute
gnomAD – Genome Aggregation Database
FAP – familial adenomatous polyposis
FDA – Food and Drug Administration (United States)
HGVS – Human Genome Variation Society
InSiGHT – International Society for Gastrointestinal Hereditary Tumours
LB – likely benign
LP – likely pathogenic
LSDB – locus-specific databases
MAF – minor allele frequency
MANE – matched annotation from NCBI and EMBL-EBI
MMR – mismatch repair
NCBI – National Center for Biotechnology Information
NGS – next-generation sequencing
NMD – nonsense-mediated decay
P – pathogenic
PM – pathogenic moderate
PP – pathogenic supporting
PS – pathogenic strong
PVS – pathogenic very strong
SNV – single-nucleotide variant
VCEP – variant curation expert panel
VUS – variant of uncertain significance

previously,[9,10] which notably includes a VCEP for *PTEN,* another established polyposis gene leading to PTEN hamartoma tumor syndrome.[11]

The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) is a multidisciplinary consortium formed in 2005 by the merger of the Leeds Castle Polyposis Group and the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer. The group established standardized variant interpretation guidelines for germline mismatch repair (MMR) variants, the underlying cause of Lynch syndrome. This led to the consistent and systematic evaluation of 2360 MMR variants independent of the ACMP/AMP framework.[12] InSiGHT also houses the world's largest curated LSDBs of variants in gastrointestinal polyposis predisposing genes on a Leiden Open Variation Database format, which currently lists 1867 different and 5628 total *APC* variants (retrieved 12/05/2022).

Building on the existing connection between InSiGHT and ClinGen, a Hereditary Colorectal Cancer/Polyposis VCEP was convened with the aim to improve accuracy and consistency in variant interpretation in *APC,* the MMR genes, and other polyposis genes, including *MUTYH* (HGNC:7257), *STK11* (HGNC:11389), *POLD1* (HGNC:9175), *POLE* (HGNC:9177), *SMAD4* (HGNC:6770), and *BMPR1A* (HGNC:1076). Here, we describe the work of the *APC* VCEP in the development of *APC*-specific classification guideline and its validation through pilot variant classification. The criteria were designed to capture disease relevance of *APC* variants in the pathogenesis of FAP but no other rare phenotypes with specific molecular mechanisms or clinical presentations (eg, gastric adenocarcinoma and proximal polyposis of the stomach [GAPPS, MONDO: 0017790] and isolated desmoids).

Methods

The *APC* VCEP

The *APC* subcommittee of the ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis VCEP (referred to here as the *APC* VCEP) consists of 46 specialists with a balanced representation of expertise, including gastroenterologists, medical geneticists, genetic counsellors, research scientists, bioinformaticians, and clinical laboratory diagnosticians. Members are from 14 countries and diverse institutions worldwide. In 3 separate monthly meetings, the *APC* VCEP devoted focused discussions in functional, computational, and clinical subgroups, which was further reviewed and synthesized in another monthly meeting with the whole committee. Overall virtual conferences were conducted over a 2-year course, and in-person meetings were held at the InSiGHT Biennial Conference in Auckland in 2019 and New Jersey in 2022.

Specification of the ACMG/AMP criteria

To provide standardized terminology and guidelines for variant classification, the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG and AMP) jointly developed criteria for pathogenic (P) and benign (B) variants based population, experimental, computational, and clinical evidence.[13] The criteria are assigned weights based on a hierarchy of benign stand-alone (BA), pathogenic very strong (PVS), benign/pathogenic strong (BS/PS), benign/pathogenic moderate (PM/BM), and benign/pathogenic supporting (PP/BP) evidence, which are combined to reach a 5-tier classification verdict ranging from pathogenic (P), likely pathogenic (LP), VUS, likely benign (LB), to benign (B) (Table 1). The assignment of evidence weight and rule combination are based on a quantitative framework using a Bayesian method, which provides statistical validation and enables further refinement of the ACMG/AMP criteria.[14] Publicly available databases, predictive tools, and published and unpublished data (experimental results, clinical laboratory data, and case-level information) were acquired through systematic literature searching and information provided by committee members. The *APC* VCEP followed the general recommendations and feedback from the ClinGen Sequence Variant Interpretation working group[15-19] and ClinGen VCEP procedures, which were further revised by results of the pilot study. The *APC*-specific criteria and any subsequent updates are available at https://cspec.genome.network/cspec/ui/svi/doc/GN089.

Selection of transcript

The preferred reference *APC* transcript for coding, intronic, and promoter 1A variants is NM_000038.6 (MANE select transcript). This transcript contains 16 exons, including a non-coding exon 1. The NM_001127510.3 transcript contains 1 additional and 1 overlapping "non-coding" exon in the 5′ region compared with NM_000038.6. For promoter 1B deletions, the preferred transcript is NM_001127511.3, which has an alternative coding exon 1. The LRG_130 summarizes all 3 "additional" exons, resulting in 18 exons (Supplemental Table 1).

Variants for pilot classification

A balanced spectrum of 58 *APC* variants were chosen from ClinVar and the InSiGHT LSDB based on the following eligibility requirements: (1) variants covering different types, such as nonsense, frameshift, splice site, missense, synonymous, intronic, stop loss, in-frame indels, and large duplications/deletions, including presumed missense or synonymous variants, which are in fact splice variants; (2) variants with conflicting interpretations within

Table 1 Rules for combining criteria *APC*-specific ACMG/AMP variant classification criteria

	Pathogenic criteria			
	Possible corresponding evidence codes			
	Very Strong	Strong	Moderate	Supporting
	PVS1	PVS1_Strong	PVS1_Moderate	PVS1_Supporting
	PS2_Very Strong	PS1	PS1_Moderate	PS3_Supporting
	PS3_Very Strong	PS2	PS2_Moderate	PS4_Supporting
	PS4_Very Strong	PS3	PS3_Moderate	PM2_Supporting
	PM6_Very Strong	PS4	PS4_Moderate	PM5_Supporting
		PM6_Strong	PM5	PM6_Supporting
		PP1_Strong	PM6	PP1
			PP1_Moderate	PP3
Combinations leading to Pathogenic classification				
1 Very Strong AND ≥ 1 Strong	1	≥1		
1 Very Strong AND ≥ 2 Moderate	1		≥2	
1 Very Strong AND 1 Moderate AND 1 Supporting	1		1	1
1 Very Strong AND ≥ 2 Supporting	1			≥2
≥ 2 Strong		≥2		
1 Strong AND ≥ 3 Moderate		1	≥3	
1 Strong AND 2 Moderate AND ≥ 2 Supporting		1	2	≥2
1 Strong AND 1 Moderate AND ≥ 4 Supporting		1	1	≥4
Combinations leading to Likely Pathogenic classification				
1 Very Strong AND 1 Moderate	1		1	
1 Very Strong AND 1 Supporting	1			1
1 Strong AND 1 Moderate		1	1	
1 Strong AND ≥2 Supporting		1		≥2
≥3 Moderate			≥3	
2 Moderate AND ≥2 Supporting			2	≥2
1 Moderate AND ≥4 Supporting			1	≥4
1 Strong AND 2 Moderate		1	2	

	Benign criteria		
	Possible corresponding evidence codes		
	Stand Alone	Strong	Supporting
	BA1	BS1	BS2_Supporting
		BS2	BS3_Supporting
		BS3	BP1
		BS4	BP2
			BP4
			BP5
			BP7
Combination leading to Benign Classification			
1 Stand Alone	1		
≥2 Strong		≥2	
Combination leading to Likely Benign Classification			
1 Strong		1	
≥2 Supporting			≥2

In addition to the original ACMG/AMP rules for combining pathogenic criteria, the following additional rules apply: (1) the combination of 1 Pathogenic Very Strong criterion and 1 Pathogenic Supporting criterion reach a classification of Likely Pathogenic; (2) the fulfillment of 1 Benign Strong criterion reaches Likely Benign; (3) if a rare variant fulfilling only PM2_Supporting but no other pathogenic codes also meets criteria for classification as (Likely) Benign, the population data are not considered conflicting and the variant can be classified as (Likely) Benign; (4) PVS1 cannot be applied in conjunction with splicing predictions (PP3) or RNA assays (PS3); (5) if RNA assay findings conflict with splice predictors, RNA findings override computational predictions (ie, BS3 over PP3 and PS3 over BP4); and (6) PS4_Variable and PP1_Variable should not be applied to a variant if BA1 or BS1 is met; however, meeting PM2_Supporting is not compulsory for pathogenic variants so that clinical criteria may be applied for such pathogenic variants with some levels of population data.

ClinVar and between ClinVar and the InSiGHT LSDB; (3) variants encompassing a broad range of criteria and different combinations of criteria; (4) variants distributed throughout the *APC* gene, including regions associated with a milder polyposis phenotype (Figure 1); and finally (5) variants with a range of classifications in ClinVar (Supplemental Table 2). Phenotype data from routine diagnostic testing were acquired for all pilot variants from

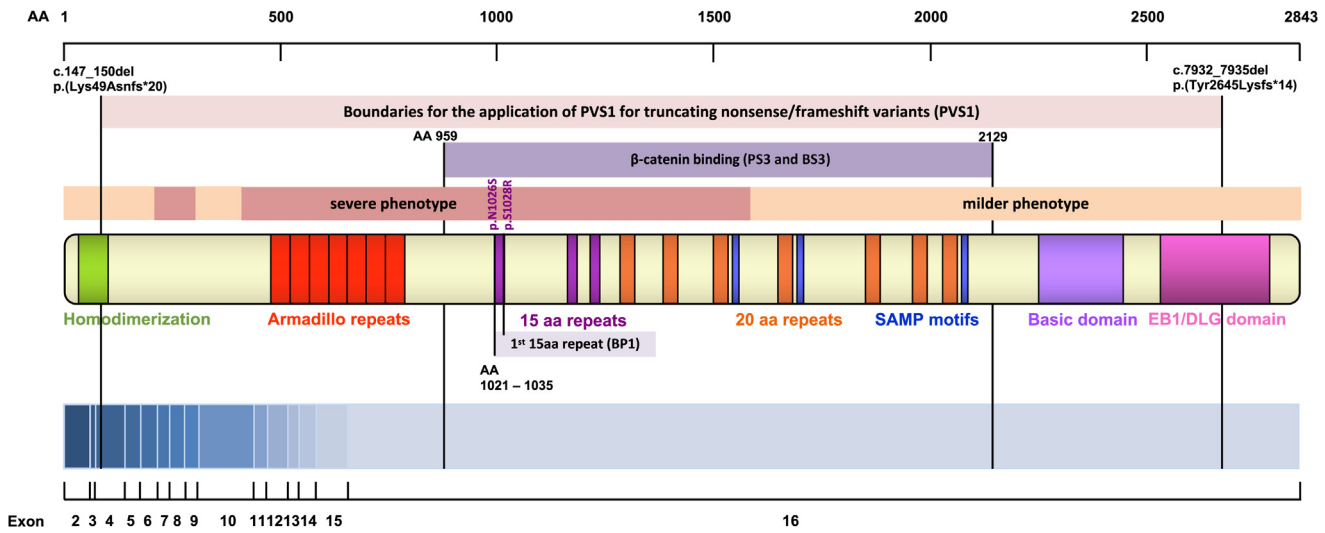


Figure 1 ***APC* gene, APC protein, and criteria boundaries and genotype-phenotype correlations**. Representation of the *APC* gene (bottom) and its main protein product (middle) on the reference sequence NM_000038.6 (non-coding exon 1 not shown). The figure shows on the top the boundaries for the application of PVS1, BS3 and BP1 and genotype-phenotype correlations. The APC protein comprises several domains and motifs as shown. The 15-aa repeats confer high-affinity binding to β-catenin, whereas the 20-aa repeats both bind and promote β-catenin phosphorylation, ubiquitination, and subsequent proteolytic degradation by a cytoplasmic destruction complex. AA, amino acid; SAMP motifs, Serine-Alanine-Methionine-Proline motifs; EB1, end-binding protein; DLG domain, discs large domain.

VCEP members and documented in a standardized, anonymized format with the bare minimum of information required for phenotypic scoring.

Each variant was independently curated by at least 2 of the 8 collaborating biocurators using ClinGen's Variant Curation Standard Operating Procedure Version 3 in the Variant Curation Interface.[20] The disparities between codes used and final classifications were examined first among the biocurators and then with the wider VCEP, enabling an iterative process by which further modifications to the evidence codes were agreed upon to enhance their usability and accuracy. The first 58 variants classified by the *APC* specifications of the ACMG/AMP criteria are now publicly accessible on ClinVar, with the designation of a 3-star review status indicating expert panel consensus and FDA recognition of evidence quality (https://www.ncbi.nlm.nih.gov/clinvar/submitters/508966/). The detailed evidence used for each curation of these *APC* variants is also available in the ClinGen Evidence Repository (https://erepo.clinicalgenome.org/evrepo/).

Results

The *APC*-specific modifications to the ACMG/AMP codes are summarized in Table 2. Further comments to all criteria are found in Supplemental Table 3, including the explanations for excluding 8 of the 28 original ACMG/AMP criteria (PM1, PM3, PM4, PP2, PP4, PP5, BP3, and BP6). For the remaining 20 criteria, gene-based and/or strength modifications were made. The rules for combining criteria to reach a final classification based on Bayesian reasoning are shown in Table 1.[14]

Minor allele frequency-driven rules (BA1, BS1, and PM2_Supporting)

The Whiffin/Ware allele frequency calculator was used to calculate *APC*-specific minor allele frequencies (MAF).[22] Assuming an estimated FAP prevalence of 1:6850 to 1:31,250 live births,[23] the value of 1:10,000 was used for the calculation of PM2_supporting. To define "allelic heterogeneity," the frequency of the most common pathogenic *APC* variant NM_000038.6:c.3927_3931del p.(Glu1309AspfsTer4) was used (0.06, found in 325 of 5527 *APC* variant records on InSiGHT LSDB, retrieved 15/12/2021). Penetrance of *APC*-associated FAP was specified as 0.9 to account for the occurrence of a milder phenotype spectrum. Based on these values, the calculated MAF suggestive of pathogenicity is ≤0.0003% (PM2_Supporting). Using an equally conservative approach, an estimated prevalence of 1:5000 people and penetrance of 0.8 were used to account for milder cases of *APC*-associated FAP in the calculation of BA1 threshold.

Depending on the severity and specificity of the phenotype, the detection rate of a pathogenic *APC* germline variant in families with colorectal adenomatous polyposis ranges between 20% to 80%.[24-26] To reflect this, "genetic heterogeneity" was set at 0.5, denoting the assumption that a (L)P *APC* variant is identified in approximately 50% of unselected patients with adenomatous polyposis. The MAF

Table 2 *APC*-specific ACMG/AMP variant classification criteria

PATHOGENIC CRITERIA		
Criteria	ACMG/AMP Description	*APC*-specific Description
PVS1_Variable	Null variant in a gene where LOF is a known mechanism of disease	As per modified decision tree (Figure 2).
PS1 PS1_Moderate	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	This criterion can be applied to both missense and splice variants. **PS1** The previously established variant was classified as Pathogenic according to the *APC*-specific modifications. **PS1_Moderate** The previously established variant was classified as Likely Pathogenic according to the *APC*-specific modifications. **Missense variants:** when the variant under assessment results in the same amino acid change as previously established (Likely) Pathogenic variant(s). There are currently only 2 Likely Pathogenic missense variants: NM_000038.6:c.3077A>G p.(Asn1026Ser) and NM_000038.6:c.3084T>A p.(Ser1028Arg). Other variants leading to the same missense change at these positions meet PS1_Moderate. No missense variant has been classified as Pathogenic based on current evidence. **Splice variants**: when the variant under assessment affects splicing at the same nucleotide as a previously established (Likely) Pathogenic variant. The splice prediction must be above defined thresholds (Supplemental Table 3) or similar to the previously established variant by multiple in silico predictors.
PS2_Variable	De novo (both maternity and paternity confirmed) in a patient with the disease and no family history	**The de novo score required for PS2_Variable is as follows**[a]:

PS2_Moderate	PS2	PS2_Very Strong
1-1.5	2-3.5	≥4

Curation of de novo score for PS2 / PM6 based on the phenotype point system (see Table 3)

	De novo score per proband	
Phenotype point per proband	De novo with confirmed parental relationships	De novo with unconfirmed parental relationships
1	2	1
0.5	1	0.5

(continued)

Table 2 Continued

<table>
<tr><th colspan="3">PATHOGENIC CRITERIA</th></tr>
<tr><th>Criteria</th><th>ACMG/AMP Description</th><th>APC-specific Description</th></tr>
<tr><td>PS3_Variable</td><td>Well established in vitro or in vivo functional studies supportive of a damaging effect</td><td>RNA assays
<table>
<tr><th>RNA event</th><th>Requirement</th><th>PS3_Variable</th></tr>
<tr><td>Premature stop codon</td><td>Absence of full-length transcript</td><td>PS3_Very Strong</td></tr>
<tr><td>Inframe skipping of exon 13 or 14</td><td><10% full-length transcript</td><td>PS3</td></tr>
<tr><td></td><td>Other[b]</td><td>PS3_Moderate</td></tr>
<tr><td>Other Inframe skipping</td><td>Absent or <10% full-length transcript</td><td>PS3_Moderate</td></tr>
<tr><td></td><td>Other[b]</td><td>PS3_Supporting</td></tr>
<tr><td>Overexpression of an alternative transcript (exons 10, 11, or 15)</td><td></td><td>PS3_Supporting</td></tr>
</table>
Protein assays
PS3_Supporting: Increased β-catenin regulated transcription activity and/or decreased binding to β-catenin by surface plasmon resonance (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of APC)</td></tr>
<tr><td>PS4_Variable</td><td>The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls</td><td>Instead of prevalence, the absolute number of affected individuals with approved phenotype points is curated to score for PS4_variable. For details regarding phenotype scoring see Table 3.
<table>
<tr><th>PS4_Supporting</th><th>PS4_Moderate</th><th>PS4</th><th>PS4_Very Strong</th></tr>
<tr><td>1-1.5</td><td>2-3.5</td><td>4-15.5</td><td>≥16</td></tr>
</table></td></tr>
<tr><td>PM1</td><td>Located in a mutational hot spot and/or well-established functional domain</td><td>N/A</td></tr>
<tr><td>PM2_Supporting</td><td>Absent/rare in controls</td><td>Rare in controls, defined by an allele frequency ≤0.0003% (0.000003) if the allele count is >1 OR by an allele frequency <0.001% (0.00001) if the allele count is ≤1.</td></tr>
<tr><td>PM3</td><td>For recessive disorders, detected in trans with a pathogenic variant</td><td>N/A</td></tr>
<tr><td>PM4</td><td>Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants</td><td>N/A</td></tr>
</table>

(continued)

Table 2 Continued

PATHOGENIC CRITERIA		
Criteria	ACMG/AMP Description	*APC*-specific Description
PM5 PM5_Supporting	Missense change at an amino acid residue where there is a different pathogenic missense change	**PM5** The reported missense variant was determined to be Pathogenic according to the *APC*-specific modifications. **PM5_Supporting** The reported missense variant was determined to be Likely Pathogenic according to the *APC*-specific modifications. There are currently only 2 Likely Pathogenic missense variants: NM_000038.6:c.3077A>G p.(Asn1026Ser) and NM_000038.6:c.3084T>A p.(Ser1028Arg). Other different missense variants at these positions meet PM5_Supporting. No missense variant has been classified as Pathogenic based on current evidence. Grantham's distance of the variant under assessment must have an equal or higher score than the reported variant.
PM6_Variable	Assumed de novo, but without confirmation of paternity and maternity	The de novo score required for PM6_Variable is as follows: PM6_Supporting: 0.5 | PM6: 1-1.5 | PM6_Strong: 2-3.5 | PM6_Very Strong: ≥4 For curation of de novo score see PS2.
PP1_Variable	Co-segregation with disease in multiple affected family members	**PP1_Strong** Variant segregates in ≥7 meioses in ≥2 families **PP1_Moderate** Variant segregates in 5 to 6 meioses in ≥1 family **PP1** Variant segregates in 3 to 4 meioses in ≥1 family
PP2	Missense variant in a gene with a low rate of benign missense variation and missense variants are a common mechanism of disease	N/A
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	**Missense variants:** Do not use computational prediction models for conservation, evolution, etc. In silico splicing predictors should be used for presumed missense variants to reveal possible splicing effects**.** **Non-canonical splice variants:** ≥2 in silico splicing predictors support a deleterious effect.
PP4	Phenotype specific for disease with single genetic etiology	N/A
PP5	Reputable source reports variant as pathogenic but the evidence is not available to perform an independent evaluation	N/A

Table 2 Continued

BENIGN CRITERIA		
Criteria	ACMG/AMP description	*APC*-specific Description
BA1	Allele frequency is >5%	GnomAD Popmax Filtering Allele frequency ≥0.1% (0.001)
BS1	Allele frequency is greater than expected for disorder	GnomAD Popmax Filtering Allele frequency ≥0.001% (0.00001)
BS2 BS2_Supporting	Observed in a healthy adult individual for a dominant (heterozygous) disorder with full penetrance expected at an early age	**BS2** ≥10 points for healthy individuals OR ≥2 times in homozygous state **BS2_Supporting** ≥3 points for healthy individuals Healthy individual — Points Age ≥ 50 years + Less than 5 adenomatous polyps in a colonoscopy + Absence of features listed in Table 3. — 1 Age ≥ 50 years + Colorectal cancer/polyposis was not the indication for testing Control, non-cancer, normal, unaffected population — 0.5
BS3 BS3_Supporting	Well established in vitro or in vivo functional studies shows no damaging effect on protein function	**RNA assays** **BS3_Supporting** RNA assay of a synonymous or intronic variant in germline patient sample demonstrates no mRNA aberration. **BS3** if, additionally, biallelic expression is shown and/or nonsense-mediated decay inhibited. **Protein assays** **BS3_Supporting** Retention of β-catenin regulated transcription activity comparable to wild type (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of *APC*).
BS4 BS4_Supporting	Lack of segregation in affected members of a family	**BS4** Affected member without the variant must score at least 1 phenotype point or at least 2 affected members without the variant must each score ≥0.5 phenotype points (see Table 3). **BS4_Supporting** Affected member without the variant must score at least 0.5 phenotype points (see Table 3).
BP1	Missense variant in gene in which only LOF causes disease	BP1 is applicable to *APC* with the exception of missense variants located in the first 15-amino acid repeat of the β-catenin binding domain (codon 1021-1035)
BP2	Co-occurrence with a pathogenic variant	Observed in trans with a (Likely) Pathogenic *APC* variant OR ≥3 times in an unknown phase with different (Likely) Pathogenic *APC* variants

(continued)

Table 2 Continued

BENIGN CRITERIA		
Criteria	ACMG/AMP description	*APC*-specific Description
BP3	In-frame deletions/insertions in a repetitive region without a known function.	N/A
BP4	Multiple lines of computational evidence suggest no impact on gene/product	**Missense variants:** BP4 is not applicable. **Synonymous (silent) or intronic variants**: ≥2 in silico splicing predictors suggest no impact on gene or gene product.
BP5	Variant in a case with an alternate molecular basis for disease.	Only applicable for an alternate genetic basis of the colorectal polyposis phenotype.
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.	N/A
BP7	A synonymous (silent) variant without predicted impact on splicing	A synonymous (silent) or intronic variant at or beyond +7/−21 for which multiple splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site.

ACMG, American College of Medical Genetics and Genomics; *AMP*, Association for Molecular Pathology; *LOF*, loss of function; *N/A*, not applicable for *APC*.

[a]Note that de novo score is distinct from phenotype points and are not equivalent to the points used to classify a variant in Tavtigian et al.[21] The parents are unaffected if they have less than 5 colorectal adenomas in a colonoscopy and are without phenotype consistent with *APC*, or they are older than 60 years of age, have no signs of gastrointestinal tumors (eg, rectal bleeding), no phenotype consistent with *APC*, and the family history is unremarkable.

[b]Reports of exon deletion/skipping/loss, insertion of intronic nucleotides.

threshold for BA1 was computed to be ≥0.006%. Because BA1 is a stand-alone criterion that yields in an uncontested Benign classification, to be even more strict, the final MAF threshold for BA1 was determined to be ≥0.1%. Similarly, based on a prevalence of 1:5000 and allelic heterogeneity of 0.06, the MAF threshold for BS1 was ≥0.001% (rounded), which is close to the MAF of the most frequent pathogenic *APC* variant c.3927_3931del; this variant was found in 2 of 236,524 alleles in the non-cancer data set from gnomAD v2.1.1 (0.0008%, retrieved 15/12/2021). This also aligned with Zastrow et al, who suggested the use of MAF of the most frequent pathogenic variant in the general population as the threshold for BS1.[27]

Computational/predictive data-driven rules (PVS1, PS1, PM5, PP3, BP4, and BP7)

Null variant in a gene in which loss of function is a known mechanism of disease (PVS1)

The majority of pathogenic *APC* variants are protein truncating (nonsense, frameshift, splice, and single/multi-exon deletions, and duplications), which leads to the disruption of β-catenin regulatory domains and subsequent loss of *APC* tumor suppressor function. The *APC* VCEP derived considerations to nonsense-mediated decay (NMD), alternative transcript, variant type, and strength-level adjustment based on known genotype-phenotype correlation (Figure 2A and Supplemental Table 3). Although NMD represents an important contributor to variant pathogenicity for other genes, it is less relevant for *APC* because its last exon (exon 16) comprises 77% of the protein (codons 653-2843), including several important functional domains (Figure 1). Indeed, truncated *APC* alleles were consistently detected in the transcript analyses of leukocyte RNA without NMD blockade.[28,29] Allele-specific expression demonstrated that premature termination in exon 16 did not trigger NMD and in other exons only partial NMD.[30,31]

Most pathogenic *APC* variants in FAP families are located in the 5′ half of the gene. Well-known statistical genotype-phenotype relationships include pathogenic variants 5′ of codon 168, between codons 312 and 412 (alternatively spliced part of exon 10), or 3′ of codon 1580, which tend to be associated with a milder (attenuated) colorectal phenotype (less or later onset adenomas), whereas pathogenic variants between codons 1250 to 1464 usually cause a severe, early-onset disease[24] (Figure 1). To reflect this, the *APC* VCEP defined the applicability of PVS1 at the extremities of the gene by evaluating the 5′-most and 3′-most variants. The variant NM_000038.6:c.147_150del p.(Lys49AsnfsTer20) was absent from population databases (PM2_Supporting), reported in 9 index patients meeting 3 phenotype points (PS4_Moderate), and segregated with FAP in 3 meioses in 2 families (PP1)[26,32-34] (unpublished data). Based on a cautious assumption that protein truncation provides only moderate evidence for pathogenicity with relative odds of 4.33:1,[14] this was the 5′-most LP variant based on available evidence (combination of 2 supporting and 2 moderate criteria). Under the same rationale, NM_000038.6:c.7932_7935del p.(Tyr2645LysfsTer14) was the 3′-most variant that could be classified as LP based on the combination of 1 strong, 1 moderate, and 1 supporting criteria. This variant was absent from gnomAD (PM2_Supporting) and reported in 9 index patients meeting 4 phenotype points (PS4)[35-39] (unpublished data). Because no truncating variant upstream/downstream of these 2 can be classified as (L)P, the current inclusive boundaries for the application of PVS1 were defined by their corresponding codons.

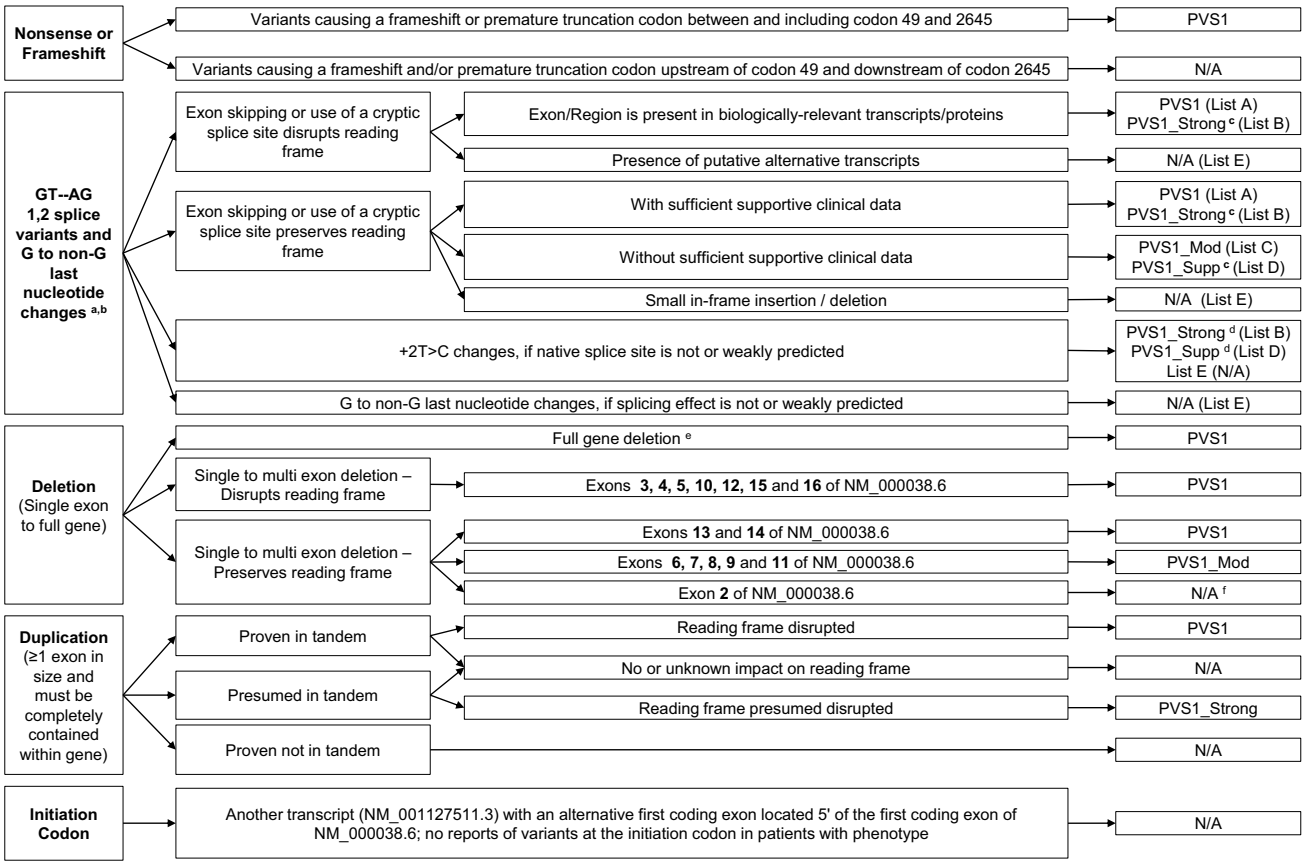
A combination of RNA analysis and splice prediction data from SpliceAI, MaxEntScan, and VarSeak were considered in the assignment of PVS1 strength to canonical ±1/2 splice single-nucleotide variants and guanine (G) to non-G change at the last nucleotide of each coding exon (Figure 2B and Supplemental Table 3). The impact on reading frame was interpreted only when the in silico predictions were concordant, and the more conservative prediction was always used unless RNA evidence was available to corroborate the prediction. Based on this, canonical ±1/2 splice variants were assigned to Lists A to E with decreasing level of evidence strength from very strong to not applicable. G to non-G last nucleotide changes were also evaluated and weighed with 1 level downgrade in strength from the corresponding canonical sites if the splicing predictions were up to the same standards.

Full-gene and frameshifting single-/multi-exon deletions fulfilled PVS1, as well as in-frame deletion of exon 13 and/or 14, in which there was convincing phenotypic data,[28] (unpublished data). Full-gene deletions were considered pathogenic by default. For other single-/multi-exon deletions with preserved reading frame, the strength level of PVS1 was downgraded to PVS1_Moderate. Proven tandem duplication with disruption of reading frame reached PVS1, whereas presumed tandem duplications only reached PVS1_Strong. Finally, because there is another transcript (NM_001127511.3) with an alternative first coding exon located 5′ of the first coding exon of NM_000038.6 and there are no reports of variants at the initiation codon in patients with relevant phenotype (internal data), PVS1 was deemed not applicable to variants affecting the initiation codon. Given the complexity in the mechanism of disease and phenotype variability of promoter variants, the VCEP did not allow the use of PVS1 for variants in the promoter region and recommend that these variants should be assessed on a case-by-case basis. To evaluate the usability of the criteria for promoter variants, an FAP-associated promoter 1B deletion was also included in the pilot study.

Missense variant in gene in which only loss of function causes disease (BP1)

Because *APC* is a gene for which primarily truncating variants are known to cause disease,[40] the missense variant type

A ***APC*** **specific modification of the PVS1 decision tree by Tayoun et al. 2018**



B ***APC*** **GT--AG 1,2 splice variants and G to non-G last nucleotide changes PVS1 strength specifications**

List A (PVS1)			List B (PVS1_Strong)	List C (PVS1_Moderate)	List D (PVS1_Supporting)	List E (N/A)	
c.136-1G>A,C,T	c.646-1G>A,C,T	c.1549-1G>A,C,T	c.220G>A,C,T	c.645+1G>A,C,T	c.729+2T>C	c.-18-1G>A,C,T	c.934-1G>A,C,T
c.136-2A>C,G,T	c.646-2A>C,G,T	c.1549-2A>C,G,T	c.422G>A,C,T	c.645+2T>A,G	c.933G>A,C,T	c.-18-2A>C,G,T	c.934-2A>C,G,T
c.220+1G>A,C,T	c.730-1G>A,C,T	c.1626+1G>A,C,T	c.834G>A,C,T	c.729+1G>A,C,T		c.135G>A,C,T	c.1313-1G>A,C,T
c.220+2T>A,C,G	c.834+1G>A,C,T	c.1626+2T>A,C,G	c.1548G>A,C,T	c.729+2T>A,G		c.135+1G>A,C,T	c.1313-2A>C,G,T
c.221-1G>A,C,T	c.834+2T>A,C,G	c.1627-1G>A,C,T	c.1548+2T>C	c.730-2A>C,G,T		c.135+2T>A,C,G	c.1408G>A,C,T
c.221-2A>C,G,T	c.835-1G>A	c.1627-2A>C,G,T	c.1626G>A,C,T	c.835-1G>C,T		c.645G>A,T,C	c.1959-1G>C,T
c.422+1G>A,C,T	c.933+1G>A,C,T	c.1743+1G>A,C,T	c.1743G>A,C,T	c.835-2A>C,G,T		c.645+2T>C	c.1959-2A>C,G,T
c.422+2T>A,C,G	c.933+2T>A,C,G	c.1743+2T>A,C,G	c.1958G>A,C,T	c.1408+1G>A,C,T		c.729G>A,T,C	
c.423-1G>A,C,T	c.1312+1G>A,C,T	c.1744-1G>A,C,T		c.1408+2T>A,C,G			
c.423-2A>C,G,T	c.1312+2T>A,C,G	c.1744-2A>C,G,T					
c.531+1G>A,C,T	c.1409-1G>A,C,T	c.1958+1G>A,C,T					
c.531+2T>A,C,G	c.1409-2A>C,G,T	c.1958+2T>A,C,G					
c.532-1G>A,C,T	c.1548+1G>A,C,T	c.1959-1G>A					
c.532-2A>C,G,T	c.1548+2T>A,G						

Figure 2 PVS1 decision tree (A) and canonical splice variant modified weights (based on reference sequence NM_000038.6) (B). [a]Splice variants must not have any detectable nearby (+/− 20 nucleotide) strong consensus splice sequence that may reconstitute in-frame splicing. [b]For details refer to Figure 2(B). PVS1_variable is applicable to listed variants only. [c]For Guanine to non-Guanine last nucleotide changes, evidence strengths are downgraded by 1 level. [d]For +2T>C changes where native splice site is not or weakly predicted, strengths are 1 level down from the other canonical ±1/2 splice variants at the same site. [e]For full gene deletions of a known haploinsufficient gene, a pathogenic classification is warranted in the absence of conflicting evidence with PVS1 alone. [f]Not applicable if promoter 1A and 1B are also deleted. NT, nucleotide; Mod, moderate; Supp, supporting; N/A, not applicable.

was regarded as evidence for benign classifications by the *APC* VCEP (BP1). The central and C-terminal domains of the APC protein are natively unfolded by bioinformatics predictions and verified experimentally by some studies, which likely explains the resistance of the APC protein to missense variation.[41] However, this criterion was not applicable to missense variants located within the first 15-amino acid repeat of the β-catenin binding domain (codon 1021-1035) because of the presence of 2 LP variants in this region: NM_000038.6:c.3077A>G p.(Asn1026Ser) and NM_000038.6:c.3084T>A p.(Ser1028Arg) (Supplemental Table 3).

Same or other amino acid change at the same position (PS1 and PM5)

The *APC* VCEP allowed the application of PS1 and PS1_Moderate for missense variants that resulted in the same amino acid change as previously established P and LP variants, respectively. Similarly, the use of PM5 and PM5_Supporting was allowed for missense variants at amino acid positions where a different missense change determined to be (L)P has been seen before. There are currently only 2 missense variants in *APC* that can be classified as LP (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)), as detailed in the explanation to PS1 in Supplemental Table 3. Other variants leading to the same missense change at these positions meet PS1_Moderate. No apparent missense variant has been classified as pathogenic based on current evidence. The *APC* VCEP further specified that PS1 and PS1_Moderate can also be used for a splice variant when it occurs at the same nucleotide position as a previously established (L)P variant and has comparable or worse splice predictions.

Protein-related in silico predictive tools (PP3, BP4, and BP7)

The large, unstructured central region of the APC protein poses unique challenge to in silico tools, which rely heavily on the accurate alignment of nucleotide sequences for the prediction of variant pathogenicity. Pathogenicity predictions by 5 protein-related computational tools (Align-GVGD, SIFT, PolyPhen2, MAPP, and REVEL) differed widely in their predictions of pathogenicity (range 17.5%-75.0%) and benignity (range 25.0%-82.5%) for *APC* missense variants in ClinVar.[42] Moreover, the predictions for the only known LP *APC* missense variants (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)) did not show an unequivocally deleterious effect across different tools. As a result, the *APC* VCEP did not recommend the use of protein-related computational prediction models (based on amino acid intrinsic features, sequence conservation, evolution, etc.) for missense variants (PP3 and BP4) at this time. However, splicing predictors should be used for presumed missense variants to reveal any splicing effect (PP3). For synonymous and intronic variants, the *APC* VCEP encouraged the use of approved splicing predictors, including SpliceAI, MaxEntScan, and varSEAK, to assess splicing, and the use of PP3/BP4 was permitted with $\geq$ 2 splicing predictors showing consistent splicing consequences. Synonymous and deep intronic variants (beyond +7/−21) variants without apparent effect on splicing could be classified as LB (BP4 and BP7).

Experimental data-driven rules (PS3 and BS3)

The *APC* gene encodes a large multifunctional protein, which is involved in several biological and developmental processes (Figure 1).[43] Germline loss-of-function variants in *APC* cause FAP through activation of the canonical Wnt/β-catenin signaling pathway.[44] Wnt/β-catenin-regulated transcription drives cell proliferation, survival, and the maintenance of an undifferentiated state, which becomes overactivated in the absence of *APC* and leads to the development of colorectal adenomas. The *APC* VCEP systematically reviewed the literature for all published functional data of *APC* variants, evaluated the validity of different types of assays used in the field, and derived gene-specific recommendations for their applicability and evidence strength level for variant classification in line with current guidelines[18,45] (Supplemental Table 4).

In the context of careful experimental design, the *APC* VCEP viewed β-catenin-regulated transcriptional assays and surface plasmon resonance binding analysis of β-catenin as acceptable supporting evidence for *APC* variant interpretation under specific circumstances. These assays were considered applicable to *APC* variants located within the β-catenin binding domain between codons 959 and 2129.[43] In addition, RNA assays in germline patient-derived samples have been well established for the detection of abnormal splicing, which represents an appreciable disease mechanism in *APC*. The strength level of RNA evidence has been modified to reflect this (Table 2).

Clinical data-driven rules (PS4, BS2, PS2, PM6, PP1, BS4, BP2, and BP5)

Increased prevalence of a variant in affected individuals compared with controls (PS4)

Because of the intra- and interfamilial variability of the colorectal phenotype, genotype-phenotype correlations, extraintestinal manifestations, and other polyposis syndromes resembling FAP, the *APC* VCEP performed a rigorous review of the available evidence and established a point-based system for scoring phenotypic information relevant to criteria PS4, PS2/PM6, and PP1 (Table 3). Given the exceedingly low allele frequency of most pathogenic *APC* variants, no case-control studies of FAP cohorts reaching statistical significance were available. The *APC* VCEP therefore defined the absolute number phenotype points required in affected individual for different PS4 strength.

Table 3 Phenotype scoring relevant to criteria PS2, PS4, PM6, PP1, and BS4 (max. 1 point per proband)

Phenotypic consistency	Phenotype highly specific for *APC*	Phenotype consistent with *APC* but not highly specific
Phenotype point per proband	1	0.5
Polyposis	Typical colorectal phenotype: 20 to 99 colorectal adenomas[a] and ≤20 y OR ≥ 100 colorectal adenomas[a] and ≤30 y OR ≥ 1000 colorectal adenomas[a] at any age OR other accepted descriptor[b] of colorectal adenomas[a] at any age	Other colorectal phenotype: ≥20 colorectal adenomas[a] at 20 to 70 y OR a documented diagnosis of FAP/AFAP OR ≥ 100/any accepted descriptor[b] of colorectal polyps without histological confirmation
Desmoid(s)	without somatic *CTNNB1* variant	Unknown *CTNNB1* status
Medulloblastoma	WNT subtype without somatic *CTNNB1* variant	Unknown subtype and/or *CTNNB1* status
Hepatoblastoma	without somatic *CTNNB1* variant	Unknown *CTNNB1* status
CHRPE	–	Multifocal/bilateral
Multiple gastric adenomas	–	Presence (≥2 gastric adenomas)
Multiple duodenal adenomas	–	Presence (≥2 duodenal adenomas)
Osteoma(s)	–	Presence
Family history	–	Typical FAP family history (dominant pedigree pattern)[c]

AFAP, attenuated FAP; *CHRPE*, congenital hypertrophy of the retinal pigment epithelium; *FAP*, familial adenomatous polyposis.

[a]Histologically confirmed adenomas, description of colorectal polyps without confirmation of histology is not accepted.

[b]Other accepted descriptors include uncountable, innumerable, countless, and carpeting, which refers to the coverage of the entire colon with distinct polyps. A single laterally spreading lesion covering a local area is not accepted.

[c]Excluded from scoring for PS2/PM6 and not applicable if PP1 is already used; can only be used if at least 1 variant carrier from the family and 1 additional relative each fulfill at least 0.5 points.

Observed in healthy adult individual (BS2)

In *APC* VCEP's terms, a healthy individual must be ≥50 years old, and either (1) had no colorectal cancer/polyposis-related indication for genetic testing or (2) had less than 5 colorectal adenomas detected in a colonoscopy but no other relevant phenotypic features (for details regarding this definition, see Supplemental Table 3). A variant heterozygote reported in a control, non-cancer, normal, or unaffected population, but lacking the above information, was counted as half a healthy individual points, thus requiring more individuals to satisfy BS2. BS2 was met when a variant was observed with ≥10 healthy individual points and BS2_Supporting with ≥3 healthy individual points. The use of the non-cancer data set of gnomAD was not considered a valid source of healthy heterozygous adult individuals because of the lack of phenotypic information (eg, insidious gastrointestinal polyps) and to avoid evidence double counting with BA1 or BS1.

Based on our knowledge, there are no reports of homozygous pathogenic germline *APC* variants in FAP patients, likely because of the lethal nature of homozygosity as observed in embryonic mouse development.[46] Therefore, the observation of a germline variant in a homozygous state ≥2 times in the non-cancer data set of gnomAD was also considered strong evidence for benign classification (BS2).

De novo data (PS2 and PM6)

APC encodes a large multifunctional protein comprising 2843 amino acids and is prone to spontaneous variation. Up to one quarter of *APC* variants occur de novo, which counteract the survival disadvantage of FAP and maintain its disease prevalence in a variant-selection balance.[47-50] Bona fide de novo occurrence was ascertained when parents lacked phenotypic features as described in Table 3. The definition of an unaffected parent was set to be more stringent than that of a healthy unaffected individual as described for BS2. Somatic and postzygotic mosaicism needs to be considered because they are frequently associated with a milder colorectal phenotype in index patients[50] and can also be present in (asymptomatic) parents.[51,52] The *APC* VCEP argued that both mosaicism in index patients and parents can be used for PS2. For low-level somatic/postzygotic mosaicism (<10%) in index patients, the presence of the variant should be confirmed in at least 1 affected tissue sample.

Co-segregation with disease in multiple affected family members (PP1) or lack of segregation (BS4)

For segregation data, family members are deemed affected if they meet at least 0.5 phenotype points as described in Table 3 or if they have ≥10 or "multiple" colorectal adenomas. Only genotype- and phenotype-positive individuals and phenotype-positive obligate heterozygotes should be included when counting meioses for PP1. Heterozygotes who have received chemoprevention may have a milder phenotype and may also be included.

When a particular variant segregates with a phenotype in a family, it provides evidence for association of the locus with the disease but not evidence for the deleteriousness of the variant itself. The pathogenicity of the variant can be

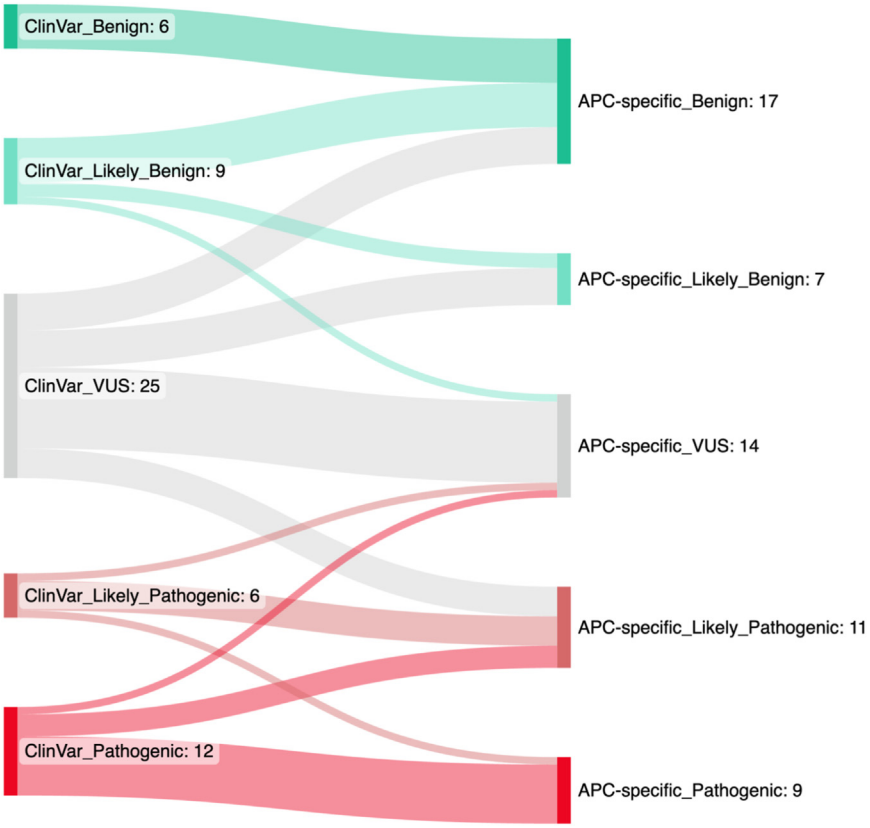


Figure 3 Classification of the 58 selected pilot *APC* variants by the original ClinVar assertion (left) and the *APC*-specific ACMG/AMP guidelines (right). VUS, variant of uncertain significance.

inferred from such evidence, with the caveat that the variant under interrogation may be in linkage disequilibrium with the true pathogenic variant in the family. Multigene panel testing and full-gene sequencing can also reduce the confounding effects of linkage disequilibrium and ascertainment bias. To qualify for lack of segregation, 1 or more affected genotype-negative members of the family must reach in total at least 1 phenotype point (BS4) or 1 genotype-negative member has at least 0.5 phenotype points (BS4_supporting).

Co-occurrence with pathogenic variants (BP2) or with alternative molecular causes for disease (BP5)

In the context of a fully penetrant dominant disorder, the detection of an *APC* variant in trans with a (L)P variant could be considered supporting evidence for benign classification. The observation of a variant in an unknown phase with ≥3 different (L)P variants would also satisfy BP2. Established genetic causes for other molecular subtypes of the colorectal polyposis phenotype include heterozygous germline variants in *POLD1* or *POLE* (polymerase-proofreading-associated polyposis), biallelic variants in *MUTYH (MUTYH*-associated polyposis), *NTHL1* (*NTHL1*-associated tumor syndrome), *MSH3*, *MBD4*, and the MMR genes *MLH1*, *MSH2*, *MSH6*, or *PMS2* (germline mismatch repair deficiency) (BP5).[50,53-58]

Validation through pilot variant classification

Representative *APC* variants ($n = 58$) were selected to encompass a range of variant types, including 25 presumed missense, 7 presumed synonymous, 8 truncating (nonsense/frameshift), 1 stop loss, 4 splice site, 7 intronic, 3 in-frame deletion/insertion variants, and 3 large deletions or duplications, including a promoter 1B deletion. Collectively, all applicable *APC*-specific ACMG/AMP codes were utilized in the classification of the pilot variants except BS4 (lack of segregation in affected relatives). The most frequently applied code was PM2_supporting ($n = 39$), which showed the rarity of *APC* variants in general. The gnomAD v2.1.1. non-cancer population database contained 17 of the pilot variants, for which either BA1 ($n = 9$) or BS1 ($n = 8$) was applied. A total of 8 institutions submitted clinical data for 50 variants, which aided in the classification of 39 variants through the application of PS4, BS2, PS2, PM6, and/or PP1. Experimental evidence was validated with corresponding codes (PS3/BS3) applied for 16 variants. PVS1 was used in

11, PS1 in 1, PM5 in 5, PP3 in 4, BP4 in 9, and BP7 in 7 variants. A list of all pilot variants, their assertions by ClinVar submitters, and their *APC* VCEP-approved classifications by the *APC* rule specifications with evidence codes applied are listed in Supplemental Table 2.

The classification of pilot variants by *APC*-specific ACMG/AMP criteria were compared with their respective classification on ClinVar, which, depending on the number and quality of submissions, could be considered as a standard for validation of gene-specific rules. There were 15 (L)B variants, 18 (L)P variants, and 25 VUS on ClinVar, which included 9 variants with conflicting assertions, defined by multiple discordant interpretations by ClinVar submitters without an overwhelming majority (≥3). Specifically, these included 2 variants with (L)B vs VUS and 7 variants with (L)P vs VUS classifications. The classification outcome of the pilot variants by the *APC*-specific rules, compared with their overall ClinVar classification, is shown in Figure 3. In summary, classification by *APC*-specific ACMG/AMP criteria was largely consistent with ClinVar classification. All 6 ClinVar B variants remained B after reclassification. 67% LB variants (6/9) were reclassified as B, whereas 1 variant NM_000038.6:c.754A>G p.(Thr252Ala) as VUS because of the paucity of clinical data. Three of the 12 P variants were downgraded to LP and 1 to VUS. One of the 6 LP variants were reclassified as P and 1 as VUS. The 3 P variants reclassified as LP were NM_000038.6:c.423-11A>G (PS3_moderate, PS4_moderate, PM2_supporting, and PP3), NM_000038.6:c.835-8A>G (PS3_moderate, PS4_moderate, PM2_supporting, and PP3), and a frameshift deletion from exons 4 to 7 (NC_000005.10:g.(?_112775619)_(112801393_?)del, PVS1 and PM2_supporting). Each of these variants were interpretated by a single submitter only in ClinVar. The strict control of evidence quality inherent to the *APC*-specific criteria may have resulted in the use of experimental and clinical codes at lower weights than ClinVar submitters and therefore a less definitive classification. Although it is worth noting that an LP classification is nevertheless possible with a compilation of evidence from different domains. In practical terms, an LP classification has a posterior probability of pathogenicity of 0.9 to 0.99, which still warrants clinical action.[14] The P variant reclassified as VUS was NM_000038.6:c.32dup p.(Gln12Alafs*Ter3) and the LP variant was NM_000038.6:c.8514C>A p.(Tyr2838Ter), which both met PS2_supporting and were located at the extremities of the protein and therefore outside of the boundaries for PVS1 application. Notably, c.32dup has been observed in heterozygous state in 3 healthy unrelated adult individuals (BS2_Variable not met; unpublished data). All 3 variants reclassified as VUS by the *APC*-specific criteria had only 1 or 2 submissions on ClinVar, which suggested the deficiency in evidence behind their initial ClinVar classification. Among the 25 VUS by ClinVar assertions, the application of the *APC*-specific criteria allowed the reclassification of 56% of the VUS (14/25) into a clinically meaningful pathogenicity class (20% each were reclassified to B and LB [5/25] and 16% to LP [4/25]). Importantly, these included the reclassification of 56% variants with conflicting interpretation (5/9).

Discussion

As the paradigm of modern genetics shifts from variant identification to interpretation, characterizing the clinical significance of variants becomes imminent for the translation of genetic testing into medical practice. The standardized terminology and guidelines developed by the ACMG/AMP provided the fundamental backbone for up-to-date variant classification but not enough granularity for the precise interpretation of variants in specific genes and diseases. At the same time, as a guideline designed to have universal applicability, some of the original ACMG/AMP criteria are unavoidably ambiguous, making it prone to subjectivity and user-to-user variability.

In this study, we assembled a multidisciplinary consortium of clinicians and scientists in relevant fields, leveraging the depth of disease expertise in the InSiGHT consortium to conduct evidence-based expert panel review of the *APC* gene using the ClinGen VCEP process. In alignment with the ACMG/AMP parent framework, the *APC*-specific variant interpretation guidelines assume a single-variant disease relationship for a high-penetrance monogenic condition. The criteria in general cannot be applied to frequent low/moderate-penetrant variants, such as NM_000038.6:c.3920T>A p.(Ile1307Lys) and NM_000038.6:c.3949G>C p.(Glu1317Gln), in which the clinical presentation and disease mechanism are more heterogenous and complex.[59,60]

The *APC* VCEP paid particular attention to ensure the mutual exclusivity of the classification codes so that the same evidence is not counted twice in the gene-specific criteria. The original ACMG/AMP codes were extended with meticulous details, especially in the clinical and experimental domains, in an effort to accurately depict the phenotypic variability of FAP and the functional diversity of the APC protein. As a medically actionable gene, the classification of an *APC* variant into either the benign or pathogenic category has important and long-lasting clinical implications. The detailed specifications for the evidence in the *APC*-specific criteria therefore serve as a quality assurance tool and reduces the risk of false-positive interpretation. At the same time, the *APC* VCEP acknowledged that certain requirements in the gene-specific criteria were quite restrictive and high-quality data might be difficult to obtain. To not dismiss evidence lightly and avoid misinterpretation of variants with clinical consequences, the VCEP also allowed strength downgrade for evidence wherever possible to accommodate for the design of cohort

studies, the data structure of reference population databases, and the set-up in routine diagnostic and screening context.

Overall, the *APC*-specific ACMG/AMP codes performed satisfactorily in the pilot study, resulting in largely consistent interpretation of well-documented benign and pathogenic variants in ClinVar, and a reclassification of 56% (14/25) of VUS into 10 (L)B and 4 LP variants. Application of the gene-specific rules help to reclassify a substantial portion of all *APC* VUS into a clinically relevant pathogenicity class, which is particularly important for the large number of VUS listed in ClinVar. Although 2 of the 18 (L)P and 1 of the 15 (L)B pilot variants were reclassified as VUS, this proportion is likely to be lower in the large group of *APC* variants because the pilot variants belonged to a selected group of variants that covered a wide range of classification scenarios and are not representative for the distribution of variants as a whole.

Although functional assessments of variants, especially RNA-based analyses, are relatively well published in the literature, the clinical data needed for classification (phenotype, proband count, segregation, and de novo status) are less well described or remained private for internal use by individual laboratories. Our work highlighted a process for standardized aggregation of case-level information from a range of different laboratories, which was paramount in the classification of the pilot variants and provided incentives for data sharing. The validity of clinical data depends heavily on the documentation of well-phenotyped individuals prepared by clinicians and genetic service providers and the competency of biocurators at analyzing phenotypic information. Establishing the infrastructure for standard variant reporting and proficient variant interpretation training would facilitate accurate and consistent application of clinical evidence. To lay the groundwork for perspective expert panel approval for the substantial number of VUS and conflicting *APC* variants submitted to ClinVar, the next step will be the design of a streamlined algorithm to systematically and comprehensively evaluate a variant and to implement this strategy in a large-scale classification approach, including the use of variant prioritization features of the ClinGen Variant Curation Interface. Prioritized lists of promising causative *APC* variants that remain at VUS will be subjected to a data mining and molecular-driven workup to collect further clinical and experimental evidence.

To resolve the interpretative challenges of variants in the post-genomic era, an *APC* subcommittee of the InSiGHT and ClinGen Hereditary Colorectal Cancer/Polyposis VCEP was constituted, and *APC*-specific variant classification criteria were developed. Future steps of the *APC* VCEP include the curation of variants in the ClinVar and InSiGHT LSDB with the outcome of an expert-panel-approved status. The *APC*-specific specifications will evolve as more evidence underlying variant pathogenicity is discovered and as the general recommendation for the ACMG/AMP guidelines from the ClinGen Sequence Variant Interpretation working group or other entities continues to develop. The most up-to-date version of the VCEP specifications are made publicly available at www.clinicalgenome.org. Moving forward, the *APC* VCEP will proceed with standardized interpretations of prioritized lists of VUS, the results of which will represent the most authoritative variant classification for widespread clinical use.

Data Availability

Data are available upon request. All variants reviewed and reclassified by the ClinGen InSiGHT Variant Curation Expert Panel in this study have been submitted to the ClinVar Database (https://www.ncbi.nlm.nih.gov/clinvar/). The detailed evidence used for the classification of these variants is available in the ClinGen Evidence Repository (https://erepo.clinicalgenome.org/evrepo/). These data may also become available upon a data transfer agreement approved by the local ethics committee and can be obtained after contacting the corresponding author (X.Y.) upon request.

Databases/URLs

ClinGen General Sequence Variant Curation Process Standard Operating Procedure: https://clinicalgenome.org/site/assets/files/7438/variant_curation_sop_v3_2_oct_2022.pdf
ClinGen Variant Pathogenicity Training Material: https://clinicalgenome.org/curation-activities/variant-pathogenicity/training-materials/
Cancer Hotspots: https://www.cancerhotspots.org
ClinGen (Clinical Genome Resource): www.clinicalgenome.org
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
HGVS (Human Genome Variation Society): https://varnomen.hgvs.org/
InSiGHT (International Society for Gastrointestinal Hereditary Tumours): https://www.insight-group.org/
APC InSiGHT LSDB (Locus-Specific Database): https://www.lovd.nl/APC
InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel: https://www.clinicalgenome.org/affiliation/50099/
Sequence Variant Interpretation Working Group: https://clinicalgenome.org/working-groups/sequence-variant-interpretation/
MaxEntScan: http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html for 5′ sites and http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html for 3′ sites
OMIM (Online Mendelian Inheritance in Man): https://www.omim.org
SpliceAI: https://spliceailookup.broadinstitute.org/

VarSeak: https://varseak.bio/
Whiffin / Ware Allele frequency calculator: http://cardiodb.org/allelefrequencyapp/

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Ethics Declaration

This study was conducted in accordance with the guidelines of the Ethics Committee of the Medical Faculty of the University of Bonn and the 1975 Declaration of Helsinki. Participants of clinical genetic testing gave written informed consent for their data to be used for clinical research and genetic investigations according to local regulations.

Conflict of Interest

SEP is a member of the scientific advisory panel of Baylor Genetics Laboratories. All other authors declare no conflicts of interest.

Additional Information

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