

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following gene: *APC*

Expert Panel Page: <https://www.clinicalgenome.org/affiliation/50099>

Gene	Disease (MONDO ID)	Transcript
<i>APC</i>	MONDO:0021056 (classic or attenuated familial adenomatous polyposis) Mode of Inheritance: Autosomal dominant inheritance	NM_000038.6

GENERAL REMARKS

The following criteria are for classic or attenuated familial adenomatous polyposis only and does not apply to Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS, [MONDO:0017790](#)).

The preferred transcript for coding, intronic and promoter 1A variants is NM_000038.6 (MANE transcript). The NM_001127510.2 transcript differs from NM_000038.6 in the number of "non-coding" exons in the 5' region, which results in different exon numbering (in NM_000038.6 there is only one non-coding exon, in NM_001127510.2 there is one additional non-coding exon and one non-coding exon overlapping with NM_000038.6; the 15 coding exons are the same). For the promoter 1B deletion the preferred transcript is NM_001127511.3, which has an alternative coding exon 1. The LRG_130 summarizes all three "additional" exons of the previously mentioned transcripts, resulting in 18 exons). To standardize, variants in this document are described in HGVS nomenclature according to their positions in the NM_000038.6 transcript unless otherwise specified. Numbered exons in this document refers to exons 1-16 in the NM_000038.6 transcript. Refer to Supplementary Table 1 for exon number conversions.

It is important to note that these criteria are not developed for low/moderate penetrant variants (e. g. c.3920T>A p.(Ile1307Lys) and c.3949G>C p.(Glu1317Gln)).

RULES FOR COMBINING CRITERIA

Important notes:

As per the SVI Working Group's recommendations, in addition to the original rules for combining pathogenic criteria, these additional rules apply for variant curation in the *APC* gene.

- 1) The combination of one Pathogenic-Very Strong criterion and one Pathogenic-Supporting criterion reach a classification of Likely pathogenic.
- 2) The fulfillment of one Benign-Strong criterion reaches Likely Benign.
- 3) If a rare variant fulfilling only PM2_Supporting but not any other pathogenic codes also meets criteria for classification as (Likely) Benign, the population data is 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 overrides computational predictions (i.e., BS3 over PP3, and PS3 over BP4).
- 6) PS4_Variable and PP1_Variable should not be applied to a variant if BS1 is met; meeting PM2_Supporting is not required so that clinical criteria may apply for pathogenic variants with some population data.

Related publication(s): PMIDs 30192042, 33348689, 4843792

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ClinGen_*APC*_ACMG_Specifications_v1

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PATHOGENIC CRITERIA																				
Criteria	APC-specific Criteria Description	Specification																		
PVS1_Variable Null variant in a gene where LOF is a known mechanism of disease	As per modified decision tree (Fig. 1 , PMID: 30192042).	Gene-specific Strength																		
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 <i>APC</i> -specific modifications. PS1_Moderate The previously established variant was classified as Likely Pathogenic according to the <i>APC</i> -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 two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and 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 ¹ or similar to the previously established variant by multiple <i>in silico</i> predictors. ¹ See Supplementary material - Evaluation of canonical ± 1 or 2 splice sites	Gene-specific Strength																		
PS2_Variable Confirmed <i>de novo</i>	The <i>de novo</i> score required for PS2_Variable is as follows: <table border="1"> <thead> <tr> <th>PS2_Moderate</th><th>PS2</th><th>PS2_Very Strong</th></tr> </thead> <tbody> <tr> <td>1</td><td>2-3</td><td>≥ 4</td></tr> </tbody> </table> For curation of <i>de novo</i> score see Tables 1 and 2 .	PS2_Moderate	PS2	PS2_Very Strong	1	2-3	≥ 4	Gene-specific Strength												
PS2_Moderate	PS2	PS2_Very Strong																		
1	2-3	≥ 4																		
PS3_Variable Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect	RNA assays <table border="1"> <thead> <tr> <th>RNA event</th><th>Requirement</th><th>PS3_Variable</th></tr> </thead> <tbody> <tr> <td rowspan="3">Premature stop codon Inframe skipping of exon 13 or 14</td><td>Absence of full-length transcript</td><td>PS3_Very Strong</td></tr> <tr> <td>< 10% full-length transcript</td><td>PS3</td></tr> <tr> <td>Other ¹</td><td>PS3_Moderate</td></tr> <tr> <td rowspan="2">Other Inframe skipping</td><td>Absent or < 10% full-length transcript</td><td>PS3_Moderate</td></tr> <tr> <td>Other ¹</td><td>PS3_Supporting</td></tr> <tr> <td>Overexpression of an alternative transcript² (exons 10, 11 or 15)</td><td></td><td>PS3_Supporting</td></tr> </tbody> </table> ¹ Reports of exon deletion/skipping/loss, insertion of intronic nucleotides 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 <i>APC</i> , PMID: 33348689)	RNA event	Requirement	PS3_Variable	Premature stop codon Inframe skipping of exon 13 or 14	Absence of full-length transcript	PS3_Very Strong	< 10% full-length transcript	PS3	Other ¹	PS3_Moderate	Other Inframe skipping	Absent or < 10% full-length transcript	PS3_Moderate	Other ¹	PS3_Supporting	Overexpression of an alternative transcript ² (exons 10, 11 or 15)		PS3_Supporting	Gene-specific Strength
RNA event	Requirement	PS3_Variable																		
Premature stop codon Inframe skipping of exon 13 or 14	Absence of full-length transcript	PS3_Very Strong																		
	< 10% full-length transcript	PS3																		
	Other ¹	PS3_Moderate																		
Other Inframe skipping	Absent or < 10% full-length transcript	PS3_Moderate																		
	Other ¹	PS3_Supporting																		
Overexpression of an alternative transcript ² (exons 10, 11 or 15)		PS3_Supporting																		

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PS4_Variable The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Instead of prevalence, the absolute number of affected individuals with approved phenotype points is defined and curated to score PS4. The phenotype points required for PS4_Variable is as follows: <table><tr><td>PS4_Supporting</td><td>PS4_Moderate</td><td>PS4</td><td>PS4_Very Strong</td></tr><tr><td>1</td><td>2-3</td><td>4-15</td><td>≥ 16</td></tr></table> For phenotype points curation see Table 1 .	PS4_Supporting	PS4_Moderate	PS4	PS4_Very Strong	1	2-3	4-15	≥ 16	Gene-specific Strength
PS4_Supporting	PS4_Moderate	PS4	PS4_Very Strong							
1	2-3	4-15	≥ 16							
PM1	Located in a mutational hot spot and/or well-established functional domain	N/A								
PM2_Supporting Absent/rare in controls	Rare in controls is defined by an allele frequency ≤ 0.0003% (0.000003).	Gene-specific Strength								
PM3	For recessive disorders, detected in trans with a pathogenic variant	N/A								
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants	N/A								
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 two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and 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 (PMID: 4843792) of the variant under assessment must have an equal or higher score than the reported variant.	Gene-specific								
PM6_Variable Assumed <i>de novo</i>	The <i>de novo</i> score required for PM6_Variable is as follows: <table><tr><td>PM6_Supporting</td><td>PM6</td><td>PM6_Strong</td><td>PM6_Very Strong</td></tr><tr><td>0.5</td><td>1</td><td>2-3</td><td>≥ 4</td></tr></table> For curation of <i>de novo</i> score see Tables 1 and 2 .	PM6_Supporting	PM6	PM6_Strong	PM6_Very Strong	0.5	1	2-3	≥ 4	Gene-specific Strength
PM6_Supporting	PM6	PM6_Strong	PM6_Very Strong							
0.5	1	2-3	≥ 4							
PP1_Variable Co-segregation with disease	PP1_Strong Variant segregates in ≥ 7 meioses in ≥ 2 families PP1_Moderate Variant segregates in 5-6 meioses in ≥ 1 family PP1 Variant segregates in 3-4 meioses in ≥ 1 family	Strength								
PP2	Missense variant in a gene that has 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. <i>In silico</i> splicing predictors should be used for presumed missense variants to reveal possible splicing effects. Non-canonical splicing variants: Multiple <i>in silico</i> splicing predictors support a deleterious effect.	Gene-specific Strength								
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 the laboratory to perform an independent evaluation	N/A								

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BENIGN CRITERIA									
Criteria	APC-specific Criteria Description	Specification							
BA1	Allele frequency $\geq 0.1\%$ (0.001)	Gene-specific							
BS1	Allele frequency $\geq 0.001\%$ (0.00001)	Gene-specific							
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 <table><tr><th>Healthy individual</th><th>Points</th></tr><tr><td>Age ≥ 50 years + Less than 5 adenomatous polyps in a colonoscopy + Absence of features in Table 1</td><td rowspan="2">1</td></tr><tr><td>Age ≥ 50 years + Colorectal cancer/polyposis was not the indication for testing</td></tr><tr><td>Control, non-cancer, normal, unaffected population</td><td>0.5</td></tr></table>	Healthy individual	Points	Age ≥ 50 years + Less than 5 adenomatous polyps in a colonoscopy + Absence of features in Table 1	1	Age ≥ 50 years + Colorectal cancer/polyposis was not the indication for testing	Control, non-cancer, normal, unaffected population	0.5	Gene-specific Strength
Healthy individual	Points								
Age ≥ 50 years + Less than 5 adenomatous polyps in a colonoscopy + Absence of features in Table 1	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 constitutional patient sample demonstrates no mRNA aberration. BS3 if additional biallelic expression is shown and/or nonsense-mediated decay inhibition was used. 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 <i>APC</i> , see PMID: 33348689)	Gene-specific Strength							
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 two affected members without the variant must each score at least 0.5 phenotype points (see Table 1). BS4_Supporting Affected member without the variant must score at least 0.5 phenotype points (see Table 1).	Gene-specific Strength							
BP1 Missense variant in gene where only LOF causes disease	BP1 is applicable to <i>APC</i> with the exception of missense variants located in the first 15-amino acid repeat of the β -catenin binding domain (codon 1021-1035)	Gene-specific							
BP2 Co-occurrence	Observed <i>in trans</i> with a (Likely) Pathogenic <i>APC</i> variant OR ≥ 3 times in an unknown phase with different (Likely) Pathogenic <i>APC</i> variants	Gene-specific							
BP3	<i>In-frame deletions/insertions in a repetitive region without a known function.</i>	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: Multiple in silico splicing predictors suggest no impact on gene or gene product.	Gene-specific							
BP5 Variant in a case with an alternate molecular basis for disease.	Only applicable for an alternate genetic basis of the colorectal polyposis phenotype.	Gene-specific							
BP6	<i>Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation.</i>	N/A							
BP7 Silent or intronic variant	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.	General							

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Table 1. Point system for phenotypic description relevant to criteria PS2, PS4, PM6, PP1 and BS4 (max. 1 point per proband)

Phenotypic consistency	Phenotype highly specific for <i>APC</i>	Phenotype consistent with <i>APC</i> but not highly specific
Phenotype point per proband	1	0.5
Polyposis	Typical colorectal phenotype: 20-99 colorectal adenomas ^a and ≤ 20 yr OR ≥ 100 colorectal adenomas ^a and ≤ 30 yr 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 yr OR a documented diagnosis of FAP / AFAP OR ≥ 100 / any accepted descriptor ^b of colorectal polyps without histological confirmation
Desmoid(s)	without somatic <i>CTNNB1</i> variant	Unknown <i>CTNNB1</i> status
Medulloblastoma	WNT subtype without somatic <i>CTNNB1</i> variant	Unknown subtype and/or <i>CTNNB1</i> status
Hepatoblastoma	without somatic <i>CTNNB1</i> variant	Unknown <i>CTNNB1</i> 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

^a histologically confirmed adenomas, description of colorectal polyps without confirmation of histology is not acceptable.

^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 acceptable.

^c excluded from scoring for PS2 / PM6 and not applicable if PP1 is already used; can only be used if at least one variant carrier from the family and one additional relative each fulfill at least 0.5 point

Abbreviations: AFAP - Attenuated familial adenomatous polyposis, CHRPE - Congenital hypertrophy of the retinal pigment epithelium, FAP - Familial adenomatous polyposis

Table 2. Curation of *de novo* score for PS2 / PM6 based on the phenotype point system*

Phenotype point per proband	<i>De novo</i> score per proband	
	<i>De novo</i> with confirmed parental relationships	<i>De novo</i> with unconfirmed parental relationships
≥ 1	2	1
0.5	1	0.5

*Note that the *de novo* score is distinct from phenotype points and are not equivalent to the points used to classify a variant according to Tavtigian et al. 2020 (PMID: 32720330).

The parents are considered unaffected if they either

- have less than five colorectal adenomas in a colonoscopy and no other phenotypic conspicuities from Table 1 OR
- are older than 60 years of age, have no signs of a gastrointestinal tumor (e. g. rectal bleeding), no phenotypic conspicuities from Table 1 and the family history is unremarkable.

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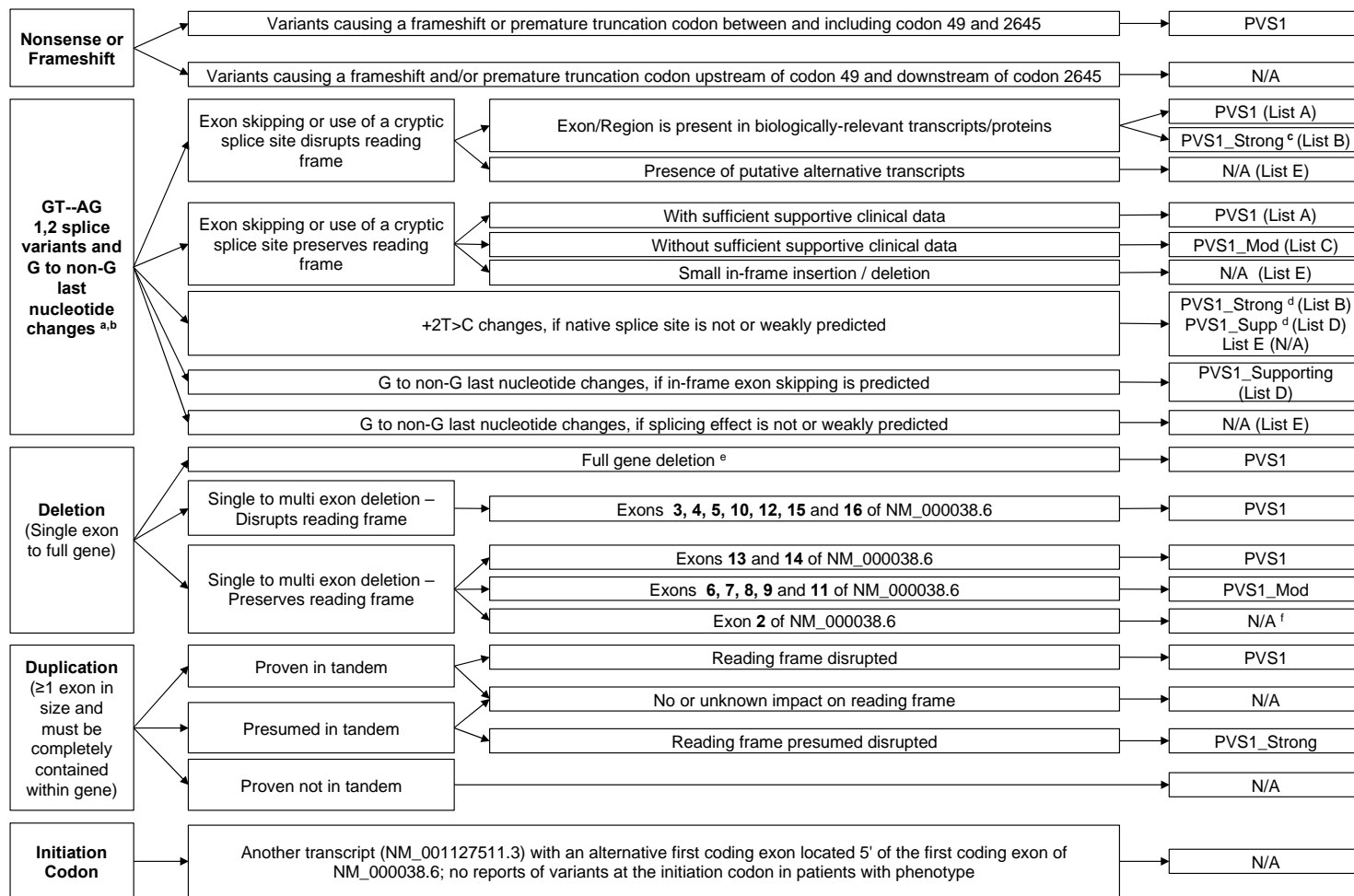
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Figure 1(A) *APC* specific modification of the PVS1 decision tree by Tayoun et al. 2018



^a Splice variants must have no detectable nearby (+/- 20 nt) strong consensus splice sequence that may reconstitute in-frame splicing.

^b For details refer to Fig 1(B). PVS1_variable is only applicable to listed variants.

^c For G to non-G last NT changes, evidence strengths are downgraded by one level.

^d For +2T>C changes for which native splice site is not or weakly predicted, strengths are one level down from the other GT-AG 1,2 splice variants at the same site Fig 1(B).

^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 1 A and 1B are also deleted.

Abbreviations: NT – nucleotide, Mod – moderate, Supp – supporting, N/A – not applicable.

Figure 1(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.221-1G>A,C,T	c.834+1G>A,C,T	c.1626+1G>A,C,T	c.1548+2T>C	c.645+1G>A,C,T	c.729+2T>C	c.-18-1G>A,C,T	c.645G>A,T,C
c.221-2A>C,G,T	c.834+2T>A,C,G	c.1626+2T>A,C,G	c.422G>A,C,T	c.645+2T>A,G	c.933G>A,C,T	c.-18-2A>C,G,T	c.645+2T>C
c.422+1G>A,C,T	c.835-1G>A	c.1627-1G>A,C,T	c.834G>A,C,T	c.729+1G>A,C,T		c.135G>A,C,T	c.729G>A,T,C
c.422+2T>A,C,G	c.933+1G>A,C,T	c.1627-2A>C,G,T	c.1548G>A,C,T	c.729+2T>A,G		c.135+1G>A,C,T	c.934-1G>A,C,T
c.423-1G>A,C,T	c.933+2T>A,C,G	c.1743+1G>A,C,T	c.1626G>A,C,T	c.730-2A>C,G,T		c.135+2T>A,C,G	c.934-2A>C,G,T
c.423-2A>C,G,T	c.1312+1G>A,C,T	c.1743+2T>A,C,G	c.1743G>A,C,T	c.835-1G>C,T		c.136-1G>A,C,T	c.1313-1G>A,C,T
c.531+1G>A,C,T	c.1312+2T>A,C,G	c.1744-1G>A,C,T	c.1958G>A,C,T	c.835-2A>C,G,T		c.136-2A>C,G,T	c.1313-2A>C,G,T
c.531+2T>A,C,G	c.1409-1G>A,C,T	c.1744-2A>C,G,T		c.1408+1G>A,C,T		c.220G>A,C	c.1408G>A,C,T
c.532-1G>A,C,T	c.1409-2A>C,G,T	c.1958+1G>A,C,T		c.1408+2T>A,C,G		c.220+1G>A,C,T	c.1959-1G>C,T
c.532-2A>C,G,T	c.1548+1G>A,C,T	c.1958+2T>A,C,G				c.220+2T>A,C,G	c.1959-2A>C,G,T
c.646-1G>A,C,T	c.1548+2T>A,G	c.1959-1G>A					
c.646-2A>C,G,T	c.1549-1G>A,C,T						
c.730-1G>A,C,T	c.1549-2A>C,G,T						

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Original ACMG/AMP criteria	Comments from the <i>APC</i> VCEP regarding <i>APC</i> -specific modifications
<p>PVS1</p> <p>Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease</p>	<p><i>APC</i> is a gene where LOF is the predominant mechanism of disease.</p> <p>Based on published transcript analyses using leukocyte RNA, truncated alleles do not (completely) undergo nonsense mediated decay (NMD) since truncated alleles are detectable without NMD blockade (Aretz et al. 2004, PMID: 15459959; Kaufmann et al. 2009, PMID: 19196998); via allele-specific expression no NMD for the last exon 16 and only a partial NMD for other exons could be detected (Castellsagué et al. 2010, PMID:20434453, Rofes et al. 2020, PMID: 33011440). Nevertheless, since truncating variants throughout the gene are well described, especially in the last exon 16 (which comprises 77% of the gene), NMD seems not to be essential for pathogenicity.</p> <p>The <i>APC</i> VCEP evaluated the evidence strength at the 5'- and the 3'-end of the <i>APC</i> gene. The first and the last truncating variants, for which additional information was available to evaluate the variants as at least likely pathogenic (using PVS1_Moderate) are c.147_150del p.(Lys49Asnfs*20) and c.7932_7935del p.(Tyr2645Lysfs*14). Using the start of the frameshift the boundaries are codon 49 and 2645. Detailed information regarding the determination of the boundaries at the 5'- and the 3'-end of the <i>APC</i> gene is given in the supplementary material at the end of this document.</p> <p>For canonical ±1 or 2 splice sites and G to non-G last nucleotide variants, splice predictions (using SpliceAI and MaxEntScan) and RNA analysis data were gathered, resulting in different weightings of PVS1 (see Fig. 1A and 1B). When convincing RNA data is available, they override in silico predictions. However, RNA analysis data was not considered necessary for the evaluation of ±1 or 2 splice sites as PVS1 (or one of the respective strength level modifications). For exons resulting in in-frame deletions or in-frame skipping available phenotype data were collected, resulting in the classification of exons 13 and 14 as applicable for PVS1. Further details and protocol for the evaluation of canonical ±1 or 2 splice sites and G to non-G last nucleotide variants is given in the supplementary material at the end of this document.</p>
<p>PS1</p> <p>Same amino acid change as a previously established pathogenic variant regardless of nucleotide change</p>	<p>Based on the current knowledge there are only two amino acid positions (1026 and 1028), where reported missense variants can be classified as Likely Pathogenic based on the <i>APC</i>-specific criteria (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)) , located in the first 15-amino acid repeat of the β-catenin binding domain (codon 1021-1035). Thus, PS1_Moderate can currently only be used for missense variants at these two amino acid positions.</p> <p>Details for the evaluation of these variants as Likely Pathogenic:</p> <ul style="list-style-type: none"> c.3077A>G p.(Asn1026Ser): based on PS3_Supporting (increased β-catenin regulated transcription activity and decreased binding to β-catenin by surface plasmon resonance, Menendez et al. 2008, PMID: 18166348), PM2_Supporting (absent in gnomAD), PP1_Strong (segregation in 10 meioses in one family and 4 meioses in another family (internal data, Menendez et al. 2008, PMID: 18166348).

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	<ul style="list-style-type: none"> c.3084T>A p.(Ser1028Arg): PS3_Supporting (increased β-catenin regulated transcription activity and decreased binding to β-catenin by surface plasmon resonance, internal data), PS4_Moderate (3 phenotype points in 6 unrelated index patients, internal data), PM2_Supporting (absent in gnomAD), PP1_Moderate (segregation in 6 meioses in two families, internal data). <p>Moreover, PS1 / PS1_Moderate can be used for splice variants when it affects splicing at the same nucleotide as a previously established (Likely) Pathogenic variant. The splice prediction must be above defined thresholds (see Supplementary material - Evaluation of canonical ± 1 or 2 splice sites) or similar to the previously established variant by multiple <i>in silico</i> predictors.</p>
PS2 <i>De novo</i> (both maternity and paternity confirmed) in a patient with the disease and no family history	<p>Modification based on SVI Recommendation for <i>De Novo</i> Criteria (PS2 & PM6) - Version 1.1 and Fortuno et al. 2020 (PMID: 33300245).</p> <p>The parents should have been colonoscoped, less than five colorectal adenomas in a colonoscopy and no other phenotypic conspicuities from Table 1 are regarded as inconspicuous. If the parents are older than 60 years and had no signs for a gastrointestinal tumor (like rectal bleeding), have no other phenotypic conspicuities from Table 1 and the family history is inconspicuous, the parents can also be regarded as unaffected.</p> <p>Be aware of somatic / postzygotic mosaicism in index patients (frequently associated with attenuated colorectal phenotype, Spier et al. 2015, PMID: 26613750) as well as in parents (Farrington et al. 1999, PMID: 9973305; Acuno-Hidalgo et al. 2015, PMID: 26054435). The VCEP suggests that both mosaicism in index patients and parents can be used for PS2. For low-level somatic / postzygotic mosaicism in index patients (<10%) the presence of the variant should be confirmed in at least one affected tissue sample.</p>
PS3 Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect on the gene or gene product	<p>Comment regarding NMD see PVS1.</p> <p>The absence of the full-length transcript or that the proportion of full-length transcript produced from the variant allele is <10% should be demonstrated for example by Sanger sequencing of the full-length fragment or allele-specific expression (Rofes et al. 2020, PMID: 33011440 and Aretz et al. 2004, PMID: 15459959).</p> <p>The overexpression of an alternative transcript should be demonstrated like it is shown for exon 15 (corresponds to coding exon 14 in the reference) in Fig. 3 by Aretz et al. 2004, PMID: 15459959 or in a comparable way.</p>
PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls	<p>Modifications are based on Fortuno et al. 2020 (PMID: 33300245).</p>
PM1 Located in a mutational hot spot and/or critical and well-	<p>Based on our knowledge there are only two amino acid positions (1026 and 1028), where reported missense variants can be classified as Likely Pathogenic based on the <i>APC</i>-specific</p>

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established functional domain (e.g. active site of an enzyme) without benign variation	<p>criteria (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)) (for details see PS1). However, until now there is not enough evidence to define this as a mutational hotspot.</p> <p>There are no other known mutational hotspots for pathogenic missense germline variants in <i>APC</i>. For somatic variants there exist a “mutation cluster region”. However, since the vast majority of somatic variants are truncating according to cancerhotspots.org, somatic variant data could not be used for <i>APC</i> (Walsh et al, 2018, PMID: 30311369).</p>
<p>PM2</p> <p>Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC</p>	<p>Based on Kelly et al. 2018 (PMID: 29300372) and the allele frequency calculator (http://cardiodb.org/allelefrequencyapp/) using the following values:</p> <p>Inheritance: monoallelic</p> <p>Prevalence: 1:10,000 (1:20,000 chromosomes)</p> <p>Allelic heterogeneity: 0.06</p> <p>Genetic heterogeneity: 1</p> <p>Penetrance: 0.9</p> <p>→ Calculated allele frequency is $\leq 0.0003\%$</p> <p>Estimates of the prevalence of FAP vary from 1:6,850 to 1:31,250 live births (based on Gene Reviews (https://www.ncbi.nlm.nih.gov/books/NBK1345/)). To be more realistic and stringent, we used an estimated prevalence of 1:10,000 for the <i>APC</i> associated FAP.</p> <p>Calculation of “Allelic heterogeneity”: frequency of the most common pathogenic <i>APC</i> variant c.3927_3931delAAAGA p.(Glu1309Aspfs*4) in <i>APC</i> LOVD (www.lovd.nl/APC): $325 / 5527 = 6\%$, query on 15/12/2021).</p> <p>To take also attenuated adenomatous polyposis cases into account, we used an estimated penetrance of 0.9.</p> <p>In general, we recommend to use the non-cancer dataset from gnomAD (v2.1.1) or other large population datasets to determine allele frequency. For populations which are underrepresented in these datasets also regional databases with ≥ 2000 tested alleles can be used (not applicable for founder populations). If a patient is from a subpopulation that has its own dataset, take that into consideration before applying PM2_Supporting.</p> <p>For indel variants you have to be careful, that the variant description / position in databases like gnomAD could be slightly different (consider also positions up- and downstream of the respective variant).</p>
<p>PM3</p> <p>For recessive disorders, detected in <i>trans</i> with a pathogenic variant</p>	<p>Not used as FAP has an autosomal dominant mode of inheritance.</p>
<p>PM4</p> <p>Protein length changes due to in-frame</p>	<p>Not used due to limited available data.</p>

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deletions/insertions in a non-repeat region or stop-loss variants	
PM5 Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	<p>Do not use if the mechanism of pathogenicity for the reported variant is a splicing defect. Multiple <i>in silico</i> splicing predictors¹ should be applied to the reported variant or the splicing defect was confirmed by RNA analysis. In this case check, if PS1 or PS1_Moderate is applicable.</p> <p>Based on the current knowledge this criterion can only be used for missense variants at the two amino acid positions 1026 and 1028, where reported missense variants can be classified as Likely Pathogenic based on the <i>APC</i>-specific criteria (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)) (for details see PS1).</p>
PM6 Assumed <i>de novo</i> , but without confirmation of paternity and maternity	<p>Modification based on SVI Recommendation for <i>De Novo</i> Criteria (PS2 & PM6) - Version 1.1 and Fortuno et al. 2020 (PMID: 33300245).</p> <p>The parents should have been colonoscoped, less than five colorectal adenomas in a colonoscopy and no other phenotypic conspicuities from Table 1 are regarded as inconspicuous. If the parents are older than 60 years and had no signs for a gastrointestinal tumor (like rectal bleeding), have no other phenotypic conspicuities from Table 1 and the family history is inconspicuous, the parents can also be regarded as unaffected.</p> <p>Be aware of somatic / postzygotic mosaicism, see also comment for PS2.</p>
PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	<p>Modifications are based on Luo et al., 2019 (PMID: 31648317), Jarvik & Browning, 2016 (PMID: 27236918). Affected individuals exhibit at least 0.5 point of the phenotype point system (see Table 1), for relatives also ≥ 10 or “multiple” colorectal adenomas are considered as 0.5 point.</p> <p>Only genotype and phenotype positive individuals and obligate carriers with phenotype are counted (note: carriers who have received chemoprevention and may have a milder phenotype can also be counted).</p> <p>Especially if cosegregation is only observed in one family it should be considered, that this observation can only give evidence that the variant or another (truly causative) variant in LD segregates with the phenotype.</p>
PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease	<p>Missense variants are not a frequent mutation type in <i>APC</i>. The majority of missense variants are regarded as benign / likely benign or VUS in ClinVar / LOVD. A few represent splice variants (Karabachev et al. 2020, PMID: 32750050) and low/moderate penetrant variants. Currently, there are only two amino acid positions with reported Likely Pathogenic missense variants (p.Asn1026 and p.Ser1028) (for details see PS1).</p>

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PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)	Modifications are based on Lee et al. 2018 (PMID: 30311375). Do not use computational prediction models (regarding conservation, evolution etc.) for missense variants, since the predictions for the only known Likely Pathogenic missense variants at positions p.Asn1026 and p.Ser1028 (for details see PS1) do not clearly predict a deleterious effect. Recommended splice prediction programs see below. ¹
PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.	Already captured by the specifications of PS4, and thus cannot be used as independent evidence.
PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation	Not used as recommended by Biesecker et al. 2018 (PMID: 29543229): Expert opinions should only be considered if accompanied by the primary evidence used.

Original ACMG/AMP criteria	Comments from the <i>APC</i> VCEP regarding <i>APC</i> -specific modifications
BA1 Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC	Allele frequency $\geq 0.1\%$ (preferably based on the non-cancer dataset from gnomAD (v2.1.1)) Based on Kelly et al. 2018 (PMID: 29300372) and the allele frequency calculator (http://cardiodb.org/allelefrequencyapp/) using the following values: Inheritance: monoallelic Prevalence: 1:5,000 (1:10,000 chromosomes) Allelic heterogeneity: 1 Genetic heterogeneity: 0.5 Penetrance: 0.8 → Calculated allele frequency is $\geq 0.006\%$ Since it is a stand-alone criterion and to be in line with other genes (Lee et al. (PMID: 30311375): <i>CDH1</i> : 0.2%; Kelly et al. (PMID: 29300372): <i>MYH7</i> : 0.1%; Luo et al. (PMID: 31648317): <i>RUNX1</i> : 0.15%), a 10-fold and rounded up value of the calculated allele frequency is used as threshold: $\geq 0.1\%$

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	<p>Estimates of the prevalence of FAP vary from 1:6,850 to 1:31,250 live births (based on Gene Reviews (https://www.ncbi.nlm.nih.gov/books/NBK1345/)). To take also attenuated and sporadic adenomatous polyposis cases into account, we used an estimated prevalence of 1:5,000.</p> <p>We used the value 0.5 as genetic heterogeneity, since in around 50% of patients with adenomatous polyposis (including attenuated cases) a (Likely) Pathogenic <i>APC</i> germline mutation can be identified; in the remaining 50% other genetic causes can be identified or the cases are unsolved so far.</p> <p>To also consider attenuated adenomatous polyposis cases, we used an estimated penetrance of 0.8 (a bit less stringent than for PM2).</p> <p>In general, we recommend to use the non-cancer dataset from gnomAD (v2.1.1) or other large population datasets to determine allele frequency. Also subpopulations can be used (with ≥ 2000 tested alleles, not applicable for founder populations), considering the highest minor allele frequency.</p>
<p>BS1</p> <p>Allele frequency is greater than expected for disorder</p>	<p>Allele frequency $\geq 0.001\%$ (preferably based on the non-cancer dataset from gnomAD (v2.1.1))</p> <p>Based on Kelly et al. 2018 (PMID: 29300372) and the allele frequency calculator (http://cardiodb.org/allelefrequencyapp/) using the following values:</p> <p>Inheritance: monoallelic</p> <p>Prevalence: 1:5,000 (1:10,000 chromosomes)</p> <p>Allelic heterogeneity: 0.06</p> <p>Genetic heterogeneity: 1</p> <p>Penetrance: 0.8</p> <p>→ Calculated allele frequency is $\geq 0.0008\%$</p> <p>Rounded value finally used: $\geq 0.001\%$</p> <p>Calculation of “Allelic heterogeneity” as for PM2.</p> <p>Prevalence and Penetrance values as for BA1.</p> <p>The calculated allele frequency of 0.0008% is equal to the MAF of the most frequent pathogenic <i>APC</i> variant (c.3927_3931delAAAGA p.(Glu1309Aspfs*4)) on gnomAD v2.1.1, this corresponds to the recommendation for BS1 by Zastrow et al., 2018 (PMID: 30311390).</p> <p>For recommendations regarding databases see BA1.</p>
<p>BS2</p>	<p>Modifications are based on Lee et al. 2018 (PMID: 30311375).</p> <p>Caveat: Phenotype is usually not visible without colonoscopy.</p>

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Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age.	The non-cancer dataset from gnomAD (v2.1.1) cannot be used for “heterozygous healthy individuals”, because of the limited phenotype information and since it is usually already used for BA1/BS1. However, the non-cancer dataset from gnomAD (v2.1.1) can be used to search for homozygous individuals.
BS3 Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing.	No comment.
BS4 Lack of segregation in affected members of a family	No comment.
BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease:	<i>APC</i> is a gene for which primarily truncating variants are known to cause disease. Based on our knowledge there are only two amino acid positions (1026 and 1028), where reported missense variants can be classified as Likely Pathogenic based on the <i>APC</i> -specific criteria (c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg)) (for details see PS1). Therefore, for all variants located in the first 15-amino acid repeat of the β -catenin binding domain (codon 1021-1035) BP1 is not allowed to be used. A number of assumed “missense” variants are in fact splice variants. At least several splice prediction tools should be used. ¹
BP2 Observed in <i>trans</i> with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in <i>cis</i> with a pathogenic variant in any inheritance pattern	Modifications are based on Mester et al. 2018 (PMID: 30311380) and Luo et al. 2019 (PMID: 31648317).
BP3 In-frame deletions/insertions in a repetitive region without a known function	<i>APC</i> VCEP: Not used due to limited available data.
BP4 Multiple lines of computational evidence	BP4 is not applicable for missense variants (comment regarding computational prediction models, see PP3). Recommended splice prediction programs see below. ¹

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suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	
BP5 Variant found in a case with an alternate molecular basis for disease	Can be used, if a (Likely) Pathogenic variant in another adenomatous polyposis gene was identified (heterozygous variants in <i>POLD1</i> or <i>POLE</i> ; biallelic variants in <i>MUTYH</i> , <i>NTHL1</i> or <i>MSH3</i> ; in patients with onset in childhood / adolescence: biallelic variants in <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> or <i>PMS2</i>). This rule is only applicable when a colorectal polyposis phenotype is present.
BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation	Not used as recommended by Biesecker et al. 2018 (PMID: 29543229): Expert opinions should only be considered if accompanied by the primary evidence used.
BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	Modifications are based on ClinGen_CDH1_ACMG_Specifications_v3: Note the <i>APC</i> rule specification does not require a conservation prediction for synonymous and intronic variants. The use of BP7 with BP4 is allowed. Recommended splice prediction programs see below. ¹

Abbreviations: FAP = Familial adenomatous polyposis, NMD = nonsense mediated decay, VCEP = variant curation expert panel, VUS = variant of unknown significance

¹ Recommended splice prediction programs:

- SpliceAI: <https://spliceailookup.broadinstitute.org/>,
- MaxEntScan : http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html for 5'splice sites and http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html for 3'splice sites
- VarSeak: <https://varseak.bio/>

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SUPPLEMENTARY MATERIAL

Determination of the cutoffs at the 5'- and the 3'-end of the *APC* gene

The *APC* VCEP evaluated the evidence strength at the 5'- and the 3'-end of the *APC* gene. The first and the last truncating variants were selected, for which additional information was available to evaluate the variants as at least likely pathogenic (using PVS1_Moderate). To evaluate the phenotype from carriers of variants at the 5'- and the 3'-end, the members of the *APC* VCEP were asked to provide available phenotypic data. Moreover, a literature / database search was performed.

1) Boundary at 5'-end:

The variant **c.147_150del p.(Lys49Asnfs*20)** meets the following criteria:

- PVS1_Moderate
- PS4_Moderate: This variant has been reported in at least nine index patients meeting at least 3 phenotype points; PMIDs 20924072, 19531215, 26681312, 31285513 and personal communication of *APC* VCEP members
- PM2_Supporting: This variant is absent from gnomAD v2.1.1
- PP1: The variant has been reported to segregate with FAP in 3 meioses from 2 families

This results in a classification as Likely Pathogenic. Based on the survey of the *APC* VCEP members and several databases (ClinVar and InSiGHT LOVD), there is no variant 5' of this variant, resulting in Likely Pathogenic.

Using the start of the frameshift variant c.147_150del p.(Lys49Asnfs*20) the boundary was set at codon 49.

2) Boundary 3'-end:

The variant **c.7932_7935del p.(Tyr2645Lysfs*14)** meets the following criteria:

- PVS1_Moderate
- PS4: This variant has been reported in at least nine index patients meeting at least 4 phenotype points; PMIDs 9824584, 1316610, 8381579, 27081525, 22135120 and personal communication of *APC* VCEP members
- PM2_Supporting: This variant is absent from gnomAD v2.1.1

This results in a classification as Likely Pathogenic. Based on the survey of the *APC* VCEP members and several databases (ClinVar and InSiGHT LOVD), there is no variant 3' of this variant, resulting in likely pathogenic.

Using the start of the frameshift variant c.7932_7935del p.(Tyr2645Lysfs*14) the boundary was set at codon 2645.

Evaluation of canonical ± 1 or 2 splice sites and G to non-G last nucleotide changes

For canonical ± 1 or 2 splice sites and last nucleotide changes splice predictions (using SpliceAI and MaxEntScan) and transcript analysis data were gathered. For SpliceAI a loss of the native splice site was considered for scores between 0.8 and 1. Of note, all canonical sites were abolished except three +2T>C alterations: c.645+2T>C (score 0.05), c.729+2T>C (score 0.4), c.1548+2T>C (score 0.3). A gain of a cryptic splice site was considered strong for scores between 0.8 and 1 and as moderate for a score between 0.2 and 0.8. For MaxEntScan predictions a score of >3 was required for credibility of a native site prediction (not fulfilled for *APC* exon 5 acceptor splice sites, but RNA analysis data shows exon skipping for several nucleotide changes at that site) and a threshold of -15% was considered for native splice site loss (Houdayer et al.

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2012, PMID 22505045). A score >3 was used as a conservative measure for cryptic site use in the context of native site loss. If they were predicted more than 20-bp away from the native splice site, they have been only considered if they were in concordance with SpliceAI and / or observed effects via RNA analysis.

If both scores showed a strong prediction for the use of a cryptic splice site, this prediction was used for the evaluation of a disruption of the reading frame or an in-frame event. If both scores showed a moderate or low score for a cryptic splice site or were conflicting and no RNA data was available, the more conservative outcome (in-frame over out-of-frame) was used. When RNA data was available, they trump predictions (if they were in conflict and the test was well designed).

For G to non-G last nucleotide changes, variants with consistently deleterious predictions by SpliceAI and MaxEntScan were assigned the PVS1 code with evidence strength one notch down from that of the canonical 1,2 variants at the same site (i.e., PVS1_Strong for the last nucleotide G to non-G change if the canonical 1,2 variants at the same site fulfill PVS1). All last nucleotide G to non-G changes were predicted to have the same splicing consequence as the canonical 1,2 variant at the same site, except c.933G>A,C,T, which causes in-frame exon skipping. This finding was also corroborated by RNA analysis. Notably the splice consequence for c.933+1G>A,C,T and c.933+2T>A,C,G were cryptic use leading to premature termination. As a result, c.933G>A,C,T were given PVS1_Supporting; the evidence strength is one level down from that of the canonical 1,2 splice variants which causes in-frame exon skipping (i.e., PVS1_Moderate).

In the following the assignment to List A-E is explained in more detail (see Fig. 1A and B):

List A (PVS1): i) Canonical splice sites for which exon skipping or use of a cryptic splice site disrupts reading frame affecting a region present in biologically relevant transcripts; ii) Canonical splice sites for which exon skipping or use of a cryptic splice site preserves reading frame and existing clinical data supports its causative role.

List B (PVS1_Strong): Splice site c.1548+2T>C variant, in which native site splice prediction is weak with SpliceAI. This variant was downgraded one level compared to the other variants at that splice site. This list also includes G to non-G last nucleotide changes with consistently deleterious splicing consequences as the canonical 1,2 splice variant affecting the same splice site.

List C (PVS1_Moderate): Canonical splice sites for which exon skipping or use of a cryptic splice site preserves reading frame and existing clinical data is not sufficient to support its causative role.

List D (PVS1_Supporting): Splice site c.729+2T>C variant, in which native site splice prediction is weak with SpliceAI. This variant was downgraded one level compared to the other variants at that splice site. This list also includes c.933G>A,C,T which causes in-frame exon skipping.

List E (N/A): i) Canonical splice sites for which exon skipping or use of a cryptic splice site disrupts reading frame affecting a region not present in biologically relevant transcripts. This concerns exon 2 [this is the first coding exon of NM_000038.6, however, there exists another transcript (NM_001127511.3) with an alternative first coding exon located 5' of exon 2 (NM_000038.6)] and exon 3 [the resulting frameshift is upstream of the 5'-boundary for truncating variants]; ii) Canonical splice sites for which use of a cryptic splice site preserves reading frame leading to a small in-frame indel; iii) Splice site c.645+2T>C variant, in which the native splice site is not predicted by SpliceAI.

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Supplementary Table 1: *APC* exon numbering (exon numbering of NM_000038.6 is used)

Start (c.)	Stop (c.)	NM_000038.6	NM_001127510.2	Coding exons ("traditional")	LRG_130	Result of exon deletion / skipping
-193	-127		1			
-126	-19		2			
-85	-19	1				
-18	135	2	3	1	4	in-frame
136	220	3	4	2	5	out-of-frame
221	422	4	5	3	6	out-of-frame
423	531	5	6	4	7	out-of-frame
532	645	6	7	5	8	in-frame
646	729	7	8	6	9	in-frame
730	834	8	9	7	10	in-frame
835	933	9	10	8	11	in-frame
934	1312	10	11	9	12	out-of-frame
1313	1408	11	12	10	13	in-frame
1409	1548	12	13	11	14	out-of-frame
1549	1626	13	14	12	15	in-frame
1627	1743	14	15	13	16	in-frame
1744	1958	15	16	14	17	out-of-frame
1959	8532	16	17	15	18	out-of-frame

Related publication(s): PMIDs 30192042, 33348689, 4843792

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