

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

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

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

	<p>(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).</p> <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>
<p>PS2</p> <p><i>De novo</i> (<u>both</u> maternity and paternity confirmed) in a patient with the disease and no family history</p>	<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>
<p>PS3</p> <p>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>	<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>
<p>PS4</p> <p>The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls</p>	<p>Modifications are based on Fortuno et al. 2020 (PMID: 33300245).</p>

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_APC_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

<p>PM1</p> <p>Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation</p>	<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 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 ≤ 0.0003%</p> <p>The allele frequency ≤ 0.0003% (0.000003) is used if the allele count is > 1. To tolerate the occurrence 1 x in gnomAD the VCEP has set an allele frequency < 0.001% (0.00001) (which is below BS1) if the allele count is ≤ 1.</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 total population from 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>

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

This document is archived and versioned on ClinGen’s website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

<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 deletions/insertions in a non-repeat region or stop-loss variants</p>	<p>Not used due to limited available data.</p>
<p>PM5</p> <p>Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before</p>	<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>
<p>PM6</p> <p>Assumed <i>de novo</i>, but without confirmation of paternity and maternity</p>	<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>
<p>PP1</p> <p>Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease</p>	<p>Modifications are based on Luo et al., 2019 (PMID: 31648317), Jarvik & Browning, 2016 (PMID: 27236918).</p> <p>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>

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

This document is archived and versioned on ClinGen’s website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

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	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).
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 Popmax Filtering Allele Frequency of 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

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

	<p>Penetrance: 0.8</p> <p>→ Calculated allele frequency is $\geq 0.006\%$</p> <p>Since it is a stand-alone criterion and to be in line with other genes (Lee et al. (PMID: 30311375): CDH1: 0.2%; Kelly et al. (PMID: 29300372): MYH7: 0.1%; Luo et al. (PMID: 31648317): RUNX1: 0.15%), a 10-fold and rounded up value of the calculated allele frequency is used as threshold: $\geq 0.1\%$</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 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 Popmax Filtering Allele Frequency of 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,</p>

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

This document is archived and versioned on ClinGen’s website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

	<p>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>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.</p>	<p>Modifications are based on Lee et al. 2018 (PMID: 30311375).</p> <p>Caveat: Phenotype is usually not visible without colonoscopy.</p> <p>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.</p>
<p>BS3</p> <p>Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing.</p>	<p>No comment.</p>
<p>BS4</p> <p>Lack of segregation in affected members of a family</p>	<p>No comment.</p>
<p>BP1</p> <p>Missense variant in a gene for which primarily truncating variants are known to cause disease:</p>	<p><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.</p> <p>A number of assumed "missense" variants are in fact splice variants. At least several splice prediction tools should be used.¹</p>
<p>BP2</p> <p>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</p>	<p>Modifications are based on Mester et al. 2018 (PMID: 30311380) and Luo et al. 2019 (PMID: 31648317).</p>
<p>BP3</p>	<p><i>APC</i> VCEP: Not used due to limited available data.</p>

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

In-frame deletions/insertions in a repetitive region without a known function	
BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	BP4 is not applicable for missense variants (comment regarding computational prediction models, see PP3). Recommended splice prediction programs see below. ¹
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/>

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

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.

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

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)]; 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.

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_APC_ACMG_Specifications_v2

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following gene: *APC*

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

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

This document is archived and versioned on ClinGen's website.

Visit <https://cspec.genome.network/cspec/ui/svi/doc/GN089> for the most recent version.

ClinGen_*APC*_ACMG_Specifications_v2