Current work of the *APC* subcommittee of the InSiGHT - ClinGen Hereditary CRC / Polyposis Variant Curation Expert Panel: Interpretation of selected *APC* variants based on the *APC*-specific ACMG/AMP variant classification criteria

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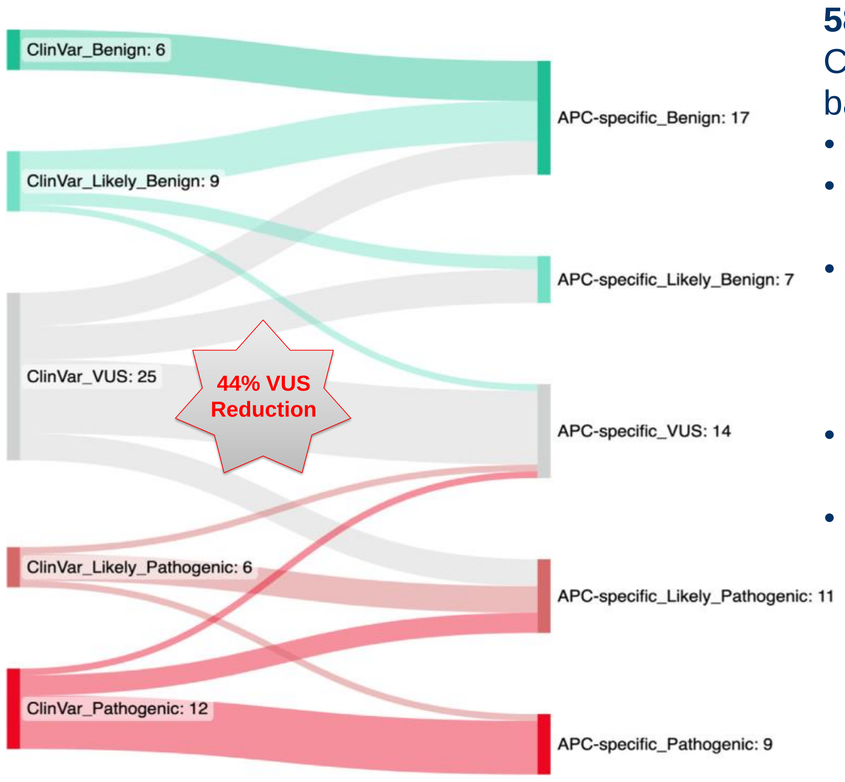
BACKGROUND: The Hereditary CRC/Polyposis Variant Curation Expert Panel was recently established by InSiGHT and ClinGen (https://clinicalgenome.org/affiliation/50099). As part of the ClinGen process, the *APC* subcommittee (***APC* VCEP**) developed **gene-specific ACMG/AMP variant classification criteria** (Spier et al. 2023; https://cspec.genome.network/cspec/ui/svi/doc/GN089, PMID: 37800450). After approval of the *APC* VCEP in December 2022, the ongoing variant assessment and curation was implemented to continue long-term variant evaluation by use of the specified criteria. All variants were first evaluated by a group of 11 biocurators, afterwards reviewed and discussed by expert members in virtual VCEP meetings, and finally made publicly available via ClinVar and the ClinGen Evidence Repository.

Main modifications in the *APC* specific ACMG/AMP variant classification criteria

- **PVS1** for truncating variants between and including codon 49 and 2645
- **Thresholds for minor allele frequency criteria:**
  - **PM2_supporting**: 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.
  - **BA1**: GnomAD Popmax Filtering Allele Frequency (AF) ≥ 0.1% (0.001)
  - **BS1**: GnomAD Popmax Filtering Allele Frequency (AF) ≥ 0.001% (0.00001)
- **Phenotype point system** (relevant for PS2/PM6, PS4 and PP1)
- **BP1** is applicable to ***APC* missense variants** (except for codons 1021-1035).
- Definition of use of **predictive** (PP3/BP4) and **experimental** data (PS3/BS3)

Pilot variant testing



58 pilot variants from ClinVar were selected based on:

- different variant types
- variants with conflicting interpretations
- variants covering a broad range and different combination of criteria
- variants distributed throughout the gene
- variants with a range of classifications

Re-evaluation of 34 "promising" *APC* variants with borderline evidence levels between uncertain significance (VUS) and (Likely) Pathogenic (LP/P)

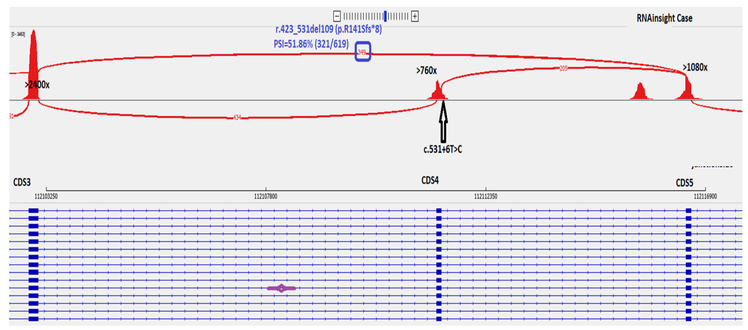
***APC* VCEP Laboratory Contributors**

Ambry Genetics · GeneDx · INVITAE · ICO Institut Català d'Oncologia · Leiden University Medical Center · MGZ Medical Genetics Center · universitätsklinikumbonn Institut für Humangenetik · Peter Mac Peter MacCallum Cancer Centre Victoria Australia

Examples of Interesting Variants

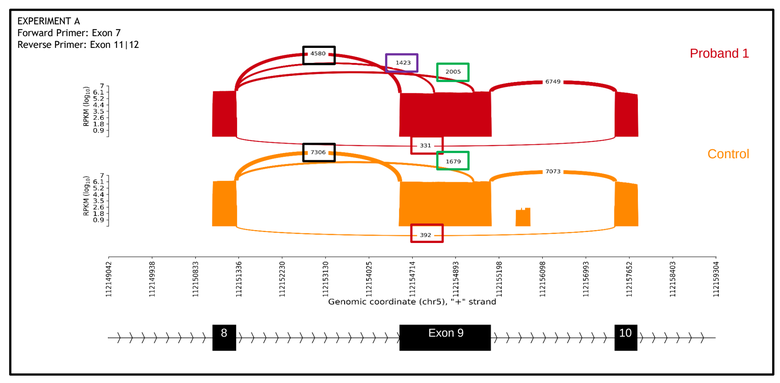
APC c.531+6T>C

- **ClinVar Classifications**: Conflicting - Pathogenic (1); Uncertain significance (1)
- **Location:** 6th nucleotide of intron 5 donor site
- ***in silico* predictions**: >2 splicing predictors indicate that this variant may affect splicing by disrupting the donor splice site of intron 5 of *APC* → **PP3**
- **RNA analysis**: skipping of exon 5 (r.423_531del109 [p.R141Sfs*8]) → **PS3_moderate** (Ambry internal data)
- **Phenotype**: 2 probands meeting phenotype score of 2 → **PS4_moderate** (Ambry internal data)
- **Population Frequency**: absent in gnomAD v2.1.1 → **PM2_supporting**
- Final Classification: **Likely Pathogenic**



APC c.1042C>T

- **ClinVar Classifications:** Conflicting - Pathogenic (1); Uncertain significance (2)
- variant seems to be a nonsense variant (p.R348Ter) located in exon 10
- **RNA analysis** (Ambry internal data):
  - in-frame aberrant transcript lacking part of exon 10 [r.934_1074del (p.V312_Q358del)]
  - increased expression of a naturally occurring alternative transcript [r.934_1236del (p.V312_Q412del), known as "exon 9a"] relative to controls
  - The predicted premature stop codon (p.R348Ter) was excluded from both the aberrant and naturally occurring transcripts and might provide a rescue mechanism for this nonsense alteration → **PVS1 not applicable**
- **Phenotype:**
  - 3 probands meeting phenotype score of 1.5 → **PS4_supporting** (PMIDs 11754114 and 12007223; CanVIG-UK)
  - 7 healthy unrelated adult individuals worth ≥ 6 healthy individual points (Ambry Genetics, Invitae, UK Biobank, 100,000 Genomes Project) → **BS2_supporting**
- **Population Frequency:** MAF is 0.0004% (1/251004 alleles) in gnomAD v2.1.1 (non-cancer) → **PM2_supporting**
- Final Classification: **VUS**



SUMMARY & CONCLUSIONS:

- **92 variants** are currently approved by the *APC* VCEP
- **~50% of ClinVar VUS** (15/31) are now B/LB/LP
- The majority (89%) of **ClinVar B/LB** (14/15) and **P/LP** variants (40/46) **retained similar classifications** by the *APC* VCEP
- Many suspicious VUS were successfully reclassified by proactively soliciting **RNA and clinical data**
- The *APC* VCEP will continue to interpret prioritised lists of **conflicting variants/VUS** to improve clinical utility.

LEGEND: ACMG = American College of Medical Genetics and Genomics; AMP = Association for Molecular Pathology; B = Benign; CRC = colorectal cancer; InSiGHT = International Society for Gastrointestinal Hereditary Tumours; LB = Likely benign; LP = Likely pathogenic, MAF = minor allele frequency, P = Pathogenic; VCEP = Variant Curation Expert Panel; VUS = variant of uncertain significance

European Reference Network for rare or low prevalence complex diseases
Network Genetic Tumour Risk Syndromes (ERN GENTURIS)

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