Curation and classification of *APC* variants in ClinVar and InSiGHT LOVD

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Background

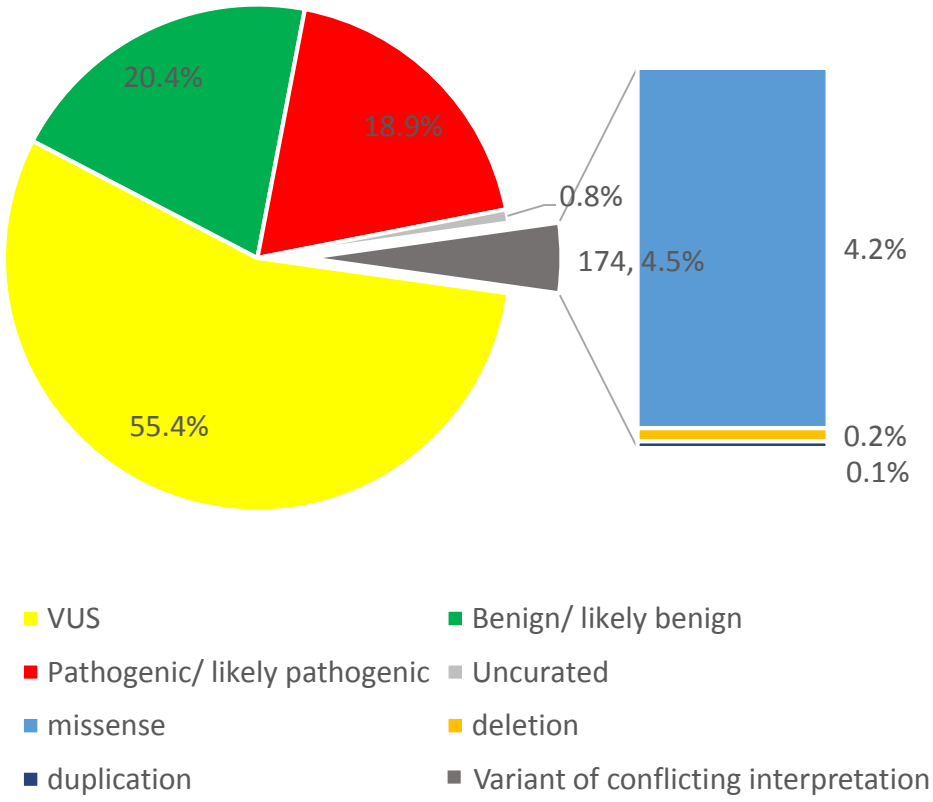
- Variant interpretation has become the bottleneck of sequencing tests
  - < 10% of 44,777 unique Variant of Unknown Significance (VUS) were reclassified over a 10-year course (Mersch et al. 2018)
- Variants with conflicting interpretations (VCIs) became commonplace
  - May have serious clinical implications for medically actionable genes

Aim

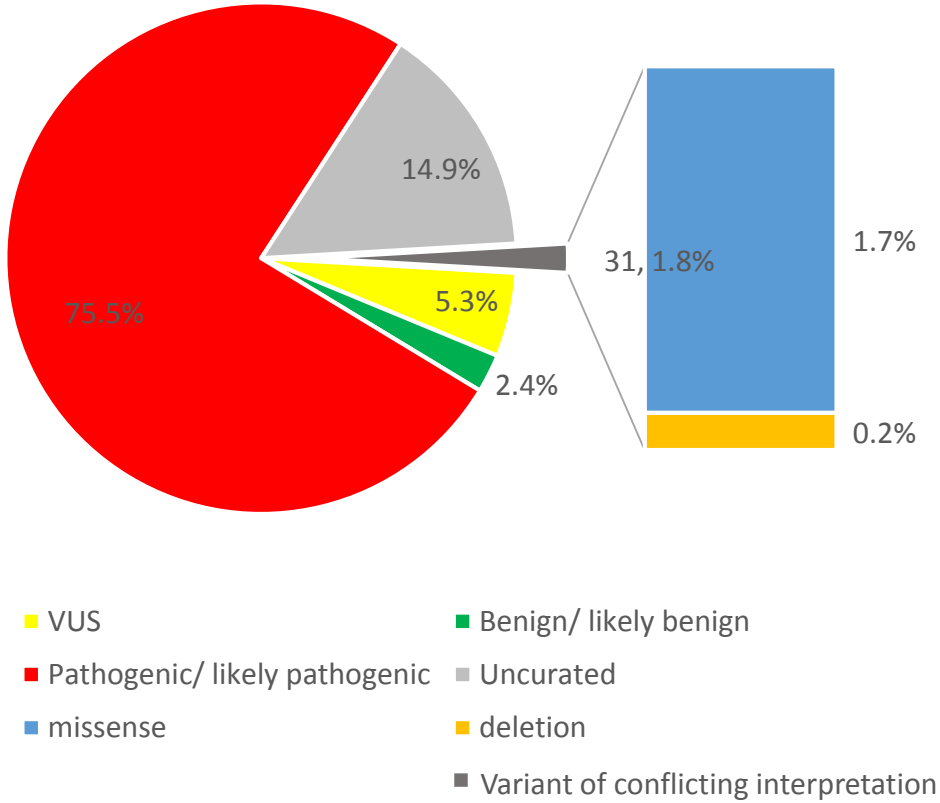
- Identify and attempt to classify *APC* variants with conflicting interpretation on ClinVar and InSiGHT databases
- Establish *APC* gene-specific criteria for the expert curation and classification by a variant interpretation committee

APC variants in ClinVar and InSiGHT databases

ClinVar: 3905 unique coding and flanking intronic sequence (+/- 20bp) *APC* variants



InSiGHT: 1691 unique *APC* variants



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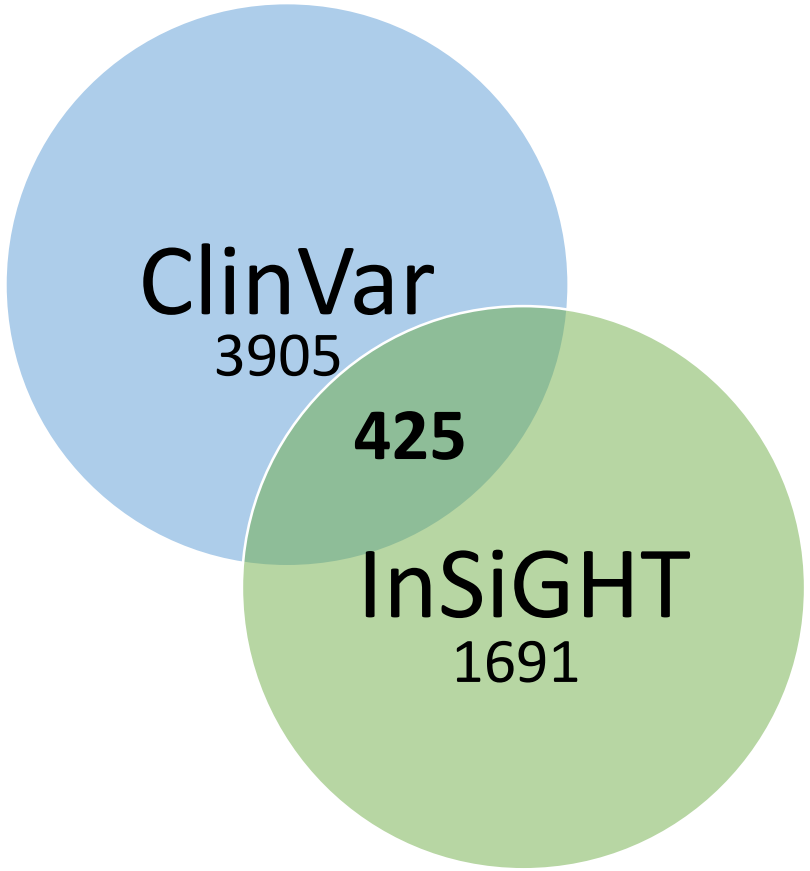
Overlap between ClinVar and InSiGHT



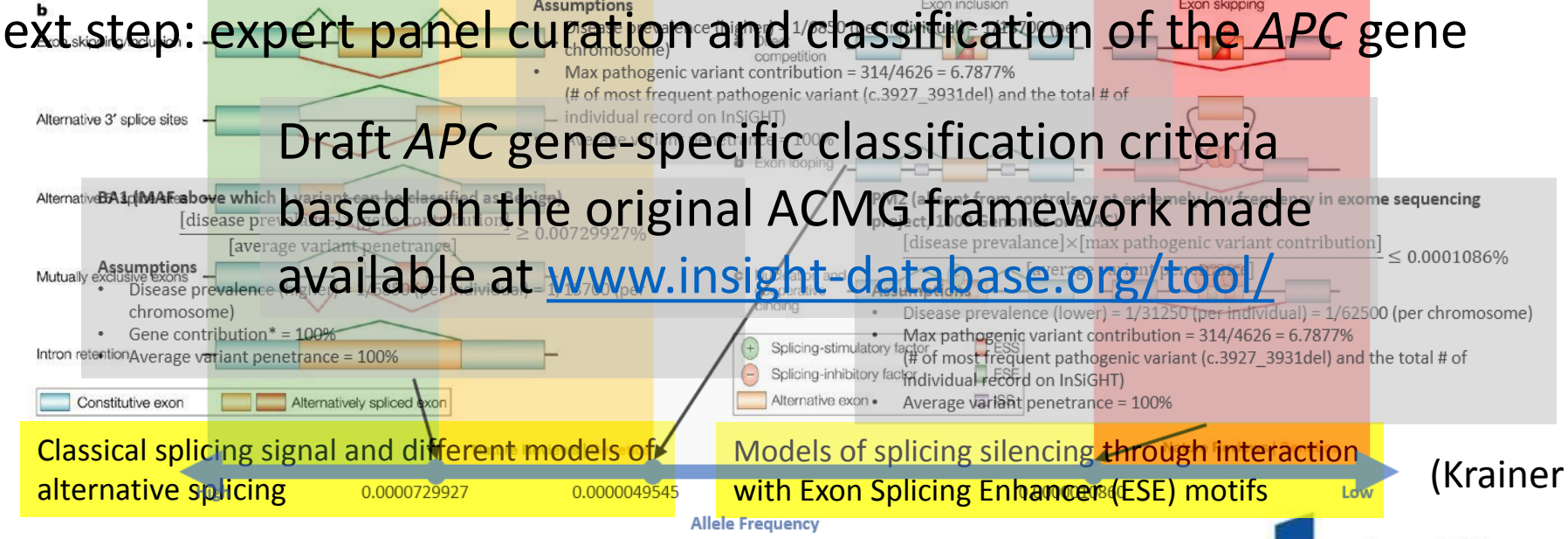
- 297 variants: consistent or largely consistent interpretations
- Remaining 138 variants
  - Uninterpreted
  - Already have inconsistent interpretation within one database
  - Conflicting interpretations between InSiGHT and ClinVar: 13

Methods

1. Applying the adapted minor allele frequency threshold from ACMG (Richards et al. 2015: Standards and guidelines for interpretation of sequence variants)
2. *In silico* analysis of predicted impact
3. Reclassification by InSiGHT ARC LOVD tool (Arbeitsgemeinschaft Bonn, Germany)
4. Literature review for transcript analysis/ functional assays
5. Next step: expert panel curation and classification of the *APC* gene

If a particular allele appears more frequent than the frequency of the disease itself, it is probably benign.

Draft *APC* gene-specific classification criteria based on the original ACMG framework made available at www.insight-database.org/tool/



Classical splicing signal and different models of alternative splicing

Models of splicing silencing through interaction with Exon Splicing Enhancer (ESE) motifs

(Krainer et al. 2002)

Results

- Based on allele frequency obtained from ExAC, GnomAD, EVS & Ensembl

	Benign: Stand-alone (MAF > 0.0073%)	Benign: Strong (MAF > 0.0005%)	Allele frequency known but do not satisfy any criteria	Pathogenic: Moderate (MAF < 0.0001086%)	Unknown allele frequency
ClinVar (168 VCIs)	49.4% (83)	28.5% (48)	4.2% (7)	0%	17.8% (30)
InSiGHT (31 VCIs)	10% (3)	0%	0%	0%	90% (21)

- 20 VCIs in InSiGHT and 6 VCIs between InSiGHT & ClinVar reclassified as Pathogenic by S. Aretz and I. Spier (Bonn, Germany)

Example:	Evidence in support of Pathogenic classification
c.802G>T p.Glu268*	Premature termination
c.423-1G>C p.(?)	Variant at canonical splice site

- Transcript analysis/ functional assays found for 11 out of the remaining variants

Example:	Evidence in support of Benign classification	Evidence in support of Pathogenic classification
c.1240C>T p.Arg414Cys	Functional assay: maintains inhibitory activity on the beta catenin/TCF4 complex as wild-type *APC* alleles	Splicing reporter minigene: p.Val313_Gln412del (Exon 9 partial deletion)

These variants will be the initial group of variants to start the work of APC Variant Interpretation Committee

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Conclusion & future perspectives

- Identified and re-classified *APC* variants on InSiGHT and ClinVar
- Will focus the attention of *APC* VIC on prioritised list of *APC* variants, who would provide the most authoritative classification of pathogenicity for widespread clinical use around the world
- We welcome any interest from students, fellows, counsellors and other professionals to assist in the variant interpretation process

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