Case Study:
Implementing ACMG guidelines on sequence variant interpretation: software-assisted variant curation and filtering

At a Glance
In this case study, you will learn:

- How Uppsala University Hospital's Molecular Genetics lab has implemented the ACMG guidelines and standards on sequence variant interpretation.

- How the Alissa Interpret facilitates automation of variant classification pipelines.

- How the ACMG classification functionality enables evaluation of all ACMG criteria on a single variant level providing a complete evidence overview.

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Introduction

Since their publication in 2015, the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) have been widely adopted. The College made these available as an educational resource for clinical laboratory geneticists to help them in the interpretation of sequence variants.

Although adherence to these standards and guidelines is voluntary and cannot replace the clinical laboratory geneticist's professional judgment, the recommendations represent a broad consensus of the clinical genetics community. With increasing volumes and the use of large gene panels (clinical, full exomes and even full genomes) in clinical genetics routine practice, labs need strong informatics tools that support them in the automation and standardization of variant assessment and reporting, in order to benefit from community standards and to keep up with the best standard of care.

"We translated the ACMG guidelines into an automated variant filtration pipeline that integrates nicely in our routine setting. When reviewing and curating variants, the software does all the time-consuming work of gathering and collating the evidence. We move straight on to reviewing the evidence and proposed classification, and confirm or modify the assessment. Our ability to implement these guidelines efficiently makes us confident we are providing the best possible quality to our referring physicians." Dr. Berivan Baskin
Uppsala University Hospital

Conclusion Summary

Uppsala University Hospital's Molecular Genetics lab illustrates how various features of the Alissa Interpret informatics module were used to implement an automated Standard Operating Procedure that reflects how the lab performs variant filtration.

Agilent Technologies

In this whitepaper, we showcase how Alissa Interpret enables labs to implement their take on the ACMG recommendations. The Molecular Genetics department at Uppsala University Hospital illustrates how it is has implemented the recommendations in their specific routine diagnostic setting, using a flexible, drag-and-drop interface to build and store the lab's variant triage protocol.

Key requirements

The standards and guidelines describe an evidence-based approach for the assessment of variants of clinically validated genes. The recommendations use literature and database-based criteria to classify variants in five different categories: benign, likely benign, uncertain significance, likely pathogenic and pathogenic. Evidence levels are weighted (e.g. "Strong", "Moderate"). To allow labs to automate their implementation of this evidence-based approach with the possibility to manually review the results, a number of specific tools are required.

Based on the guidelines we have grouped the 28 criteria into 11 categories where for each category the corresponding evidence is given to optimally support the variant assessment process. Depending on the lab specialists' acceptance of criteria, a classification will be predicted according to the scoring matrix as proposed in Richards et al., 2015. The possibility to take along the variant classifications and summaries based on the ACMG criteria, combined with Alissa Interpret's flexible analysis approach provides a time-efficient way to address variant management and curation challenges.

Annotation sources, such as population, disease-specific, and sequence databases

The guidelines recommend the use of a wide range of criteria. Examples include: population databases such as the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/), Disease databases such as ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and sequence databases such as RefSeq (http://www.ncbi.nlm.nih.gov/refseq/rsg). With Alissa's Interpret platform, labs can integrate and use a wide range of community-accepted resources, including the tools and data.sources recommended in the ACMG guidelines. Moreover, with Alissa Interpret, labs can benefit from full version control and traceability on these resources.

Rules for combining criteria to classify sequence variants: automation of decision trees and scoring combined with variant review to manually accept ACMG criteria

The guidelines recommend a broad set of informative criteria for assessing the clinical impact of a sequence variant. With each criterion, they also provide a level of evidence strength. For example, for a de novo variant in a patient with the disease, no family history and with both maternity and paternity confirmed, the evidence to classify the variant as 'Pathogenic' is suggested to be 'Strong'. Other levels are 'Very Strong', 'Moderate' and 'Supporting'. The guidelines also propose a scheme of rules by which labs can combine different criteria for classifying variants with different levels of evidence. In order to automate such a scheme, a tool set is required to represent rules into a workflow, and associate scores to variants accordingly. Alissa Interpret elegantly provides such a system by means of classification trees. The user can choose from a library of filter components that each represent filter criteria such as 'population frequency', and can drag-and-drop these into a decision tree.
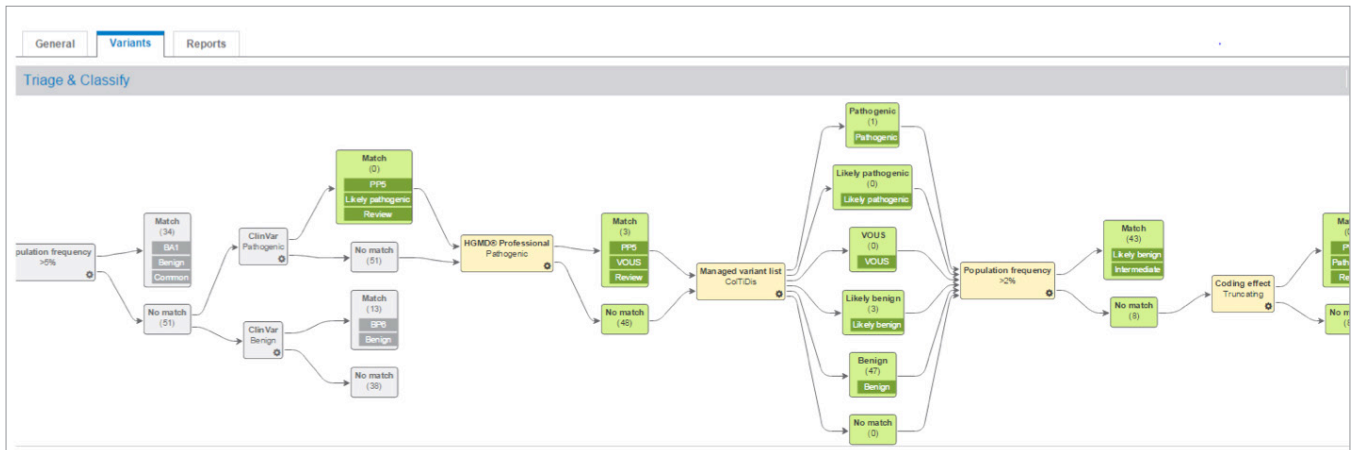
Triage and classify variants with CLASSFICATION TREES

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MANUALLY ACCEPT ACMG CRITERIA for each variant based on available evidence

	Effect	✓ 1 ✗ 5 ? 0

| Code	Description	Evaluation
PVS1	null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease **Notes** *Click to add notes*	✓ ✗
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 **Notes** *Click to add notes*	✓ ✗

Variant information

Position	1:237,711,797
Type	snp
Location	exonic
Exon	26
Effect	synonymous

Variant Links

dbSNP Variant (rs2253273)
PubMed search
Google search
ClinVar search
COSMIC (COSM4143550)

Classification [Pathogenic ▼]

Include in report [- select - ▼]

REVIEW PROPOSED CLASSIFICATION

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INCLUDE VARIANT IN REPORT

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ADD TO MANAGED VARIANT LIST (MVL)

Postnatal curated variants

NM_001035.2 c.2973A>G

Gene	RYR2
Transcript	NM_001035.2
cDNA	c.2973A>G
Protein	p.Ser991=
Type	snp
Location	exonic
Exon	26
Effect	synonymous

Classification [Benign ▼]

ACMG evaluation
Accepted criteria: BA1 - BP7
Rejected criteria: PVS1 - PM2 - PM4 - PP2 - PP3 - BP1 - BP3

Variant information

| B I U x₂ x² A▾ Iₓ | Size ▾ | ▦ ⛓ ⛓ | ≔ ≔ ⇥ ⇤ | ↩ ↪ | ⤢ |

Based on the ACMG standards and guidelines, this variant is classified as Benign.
Following criteria are met:
BA1 - Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
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

Report abstract

| B I U ↩ ↪ ⤢ |

ACMG Guidelines

RYR2   c.2973A>G   p.Ser991=

ACMG classification ❶   Benign

Evaluation		
▸ Effect		✓ 1 ✗ 5 ❷ 0
▸ Population		✓ 1 ✗ 1 ❷ 3
▸ Computational evidence		✓ 0 ✗ 1 ❷ 1
▸ Position		✓ 0 ✗ 0 ❷ 2
▸ External databases		✓ 0 ✗ 0 ❷ 2
▸ Inheritance		✓ 0 ✗ 0 ❷ 4
▸ Cis/trans		✓ 0 ✗ 0 ❷ 2
▸ Functional studies		✓ 0 ✗ 0 ❷ 2
▸ Location		✓ 0 ✗ 0 ❷ 1
▸ Phenotype		✓ 0 ✗ 0 ❷ 1
▸ Alternate molecular basis		✓ 0 ✗ 0 ❷ 1

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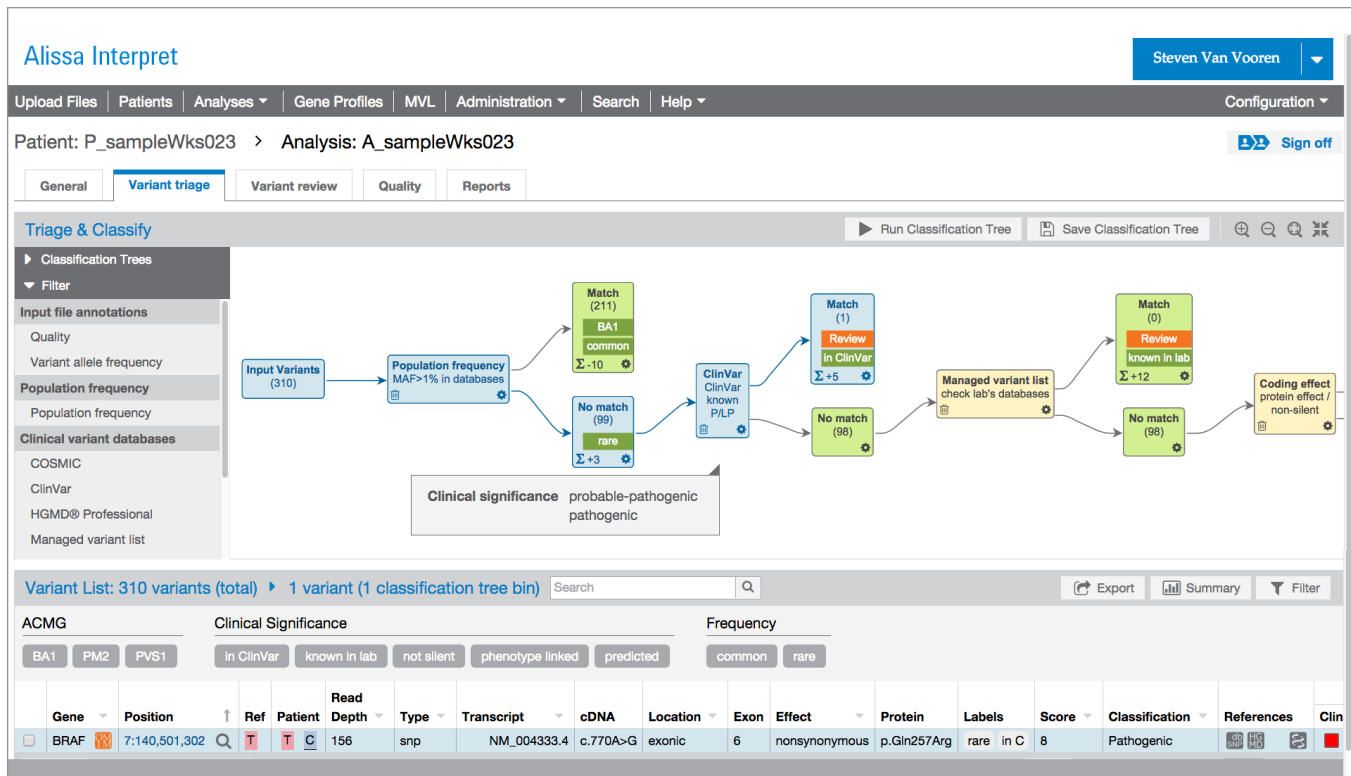
Figure 2. An example decision tree. This tree shows how a number of variants are checked against common population databases, and - based on a specific Minor Allele Frequency- are labeled as "benign" with ACMG category "BA1", as per the recommendation to assert an allele frequency over 5% in the Exome Sequencing Project, the 1000 Genomes Project, or the Exome Aggregation Consortium. The path a selected variant follows through the tree is highlighted. As a variant is filtered through the tree, it picks up annotations, classifications, scores and labels as configured by the lab.

After the classification tree has been executed, variants marked for review can be manually curated. This means the ACMG criteria can be evaluated to be accepted or rejected for each variant and based on the accumulated evidence a classification (eg. likely pathogenic) can be assigned to this particular variant.

In **Figure 3**, an overview of the ACMG classification criteria is given for the p.Trp1105Ser variant. At a glance, we can see the labels that were associated to this variant by the decision tree while within the ACMG Guidelines tab, we can get insight into the different evaluation criteria accompanied by the relevant evidence summaries. When evaluating for example the computational evidence, we can decide to accept the PP3 criterium for this variant by taking into account the outcomes from multiple functional effect prediction algorithms, conservation and evolutionary splicing impact. At the bottom of figure 3, we can immediately see the majority of outcomes is shown in red and therefore the overall prediction is (probably) damaging. Besides the color coding the score ranges can also be visualized on hover and together they enable a quick and easy evaluation to accept the PP3 criterium for computational evidence.

When completing the evidence assessment for the ACMG guidelines a variant classification will be proposed.

In **Figure 3**, after accepting the PP3 criterion and rejecting BP4, a temporary VOUS classification is proposed. If this classification seems justified, it can be copied to the variant's classification right away and if desired a summary can be copied to the variant's assessment. Moreover, if this variant would have been observed previously and an ACMG scoring had been performed, its classification can easily be retrieved, without the need to go through the same process again.

Figure 3. The ACMG variant classification tab provides insight into the different evaluation criteria accompanied by the relevant evidence summaries. Based on the provided evidence, ACMG criteria can be accepted or rejected. When completing the evidence assessment for the ACMG guidelines a variant classification will be proposed.

Implementation

The molecular genetics laboratory at the Uppsala University Hospital has implemented the ACMG guidelines using Alissa Interpret and validated their approach on a set of clinical cases. The lab has implemented different criteria as well as levels of evidence in a decision tree, partially shown in **Figure 4**. In this view, a validated pipeline is run on a Connective Tissue Panel sample, showcasing a variant in the COL1A2 gene that is reported as clinically relevant. The protocol represented by the tree has checked all variants in the assay, and highlighted the p.Gly949Ser variant for review. The clinical geneticist consecutively verifies relevant sources – in this case: ESP, 1000 Genomes, ExAC, HGMD, in silico score annotations from ACMG-recommended SIFT, Mutation Taster and PolyPhen, and a confirmed spectrum of missense mutations in the gene at hand. Parental samples tested negative for this variant.
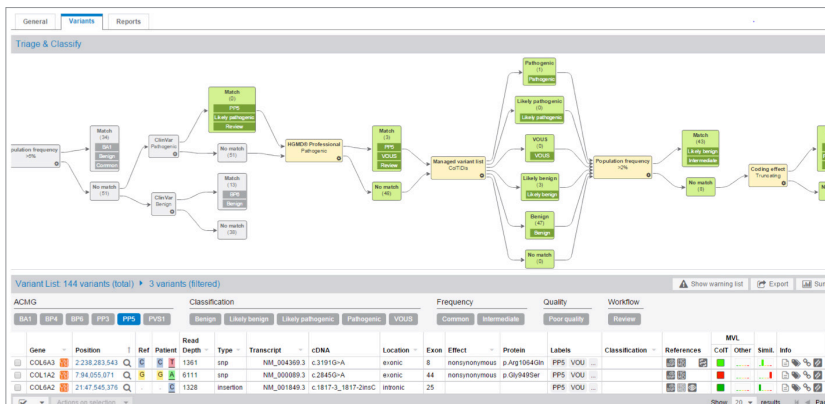
Figure 4. Partial view of the Uppsala University Hospital decision tree representing their filtration strategy, investigating public and in-house variant databases, modes of inheritance, population frequency statistics databases, and variant coding effect. Top: decision tree. Middle: currently selected ACMG category PP5. Bottom: variants matching selected criteria. (Courtesy of Dr. Berivan Baskin)

Conclusion

With this case study, the lab has illustrated how various features of Alissa Interpret were used to implement an automated Standard Operating Procedure that reflects how the lab performs variant filtration. This case illustrates strong advantages in lab efficiency - whereas a manual process of variant filtration is time consuming and error prone, the lab benefits from automation of these manual protocols, freeing up time for genetic specialists to focus on variant interpretation and reporting while being able to manually review flagged variants and their associated ACMG criteria with supporting evidence. The possibility to copy the ACMG assessment from previous observations further endorses the lab specialist to avoid double work and focus on new observations. To conclude, this approach provides an elegant solution for labs to save time by automating their protocols while keeping control over the decision-making process via a dedicated variant review section.

Intended Use Statement

Alissa Interpret software is intended for variant storage, visualization, and annotation using public, commercial and customer internal data sources. It allows end users to set up pipelines to perform or automate the triage and classification of genetic variants. It provides features for recording variant assessments and the drafting of variant analysis reports. The integration capabilities allow for the automated exchange of variant and report information with external software systems.

Alissa Interpret software is intended to be used by trained lab professionals, clinical geneticists and molecular pathologists as a decision-support software platform for the analysis and interpretation of genetic variants identified in human samples in the context of clinical information recorded for a sample.

LEARN MORE:

www.agilent.com/lifesciences/alissa

Email us at **cad.info@agilent.com**

Trusted Answers. Together.

Alissa Interpret is a USA Class I Exempt Medical Device, Europe CE IVD, Canada and Australia Class I IVD Device.

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