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Case Study:

An Efficient Clinical Pipeline for Microcephaly, RASopathy and Leukodystrophy Gene Panels Using Alissa Interpret's Flexible Classification Functionality: The Hôpital Robert-Debré Experience

At a Glance

In this case study, you will learn:

- How the molecular geneticists at the Robert-Debré hospital save time by using Alissa Interpret to set up a variant classification strategy that helps diagnose patients suffering from rare developmental pathologies.
- How Alissa Interpret can be used in a flexible way, through the extensive labeling and variant review functionality.
- How Robert-Debré strengthens its clinical pipeline by using its patient population statistics
- How the molecular lab at Robert-Debré has built a valuable knowledge base using the Alissa Interpret Managed Variant List (MVL) capabilities.

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Introduction

Combining gene panels with next generation sequencing (NGS) has provided a fast and cost-efficient way to assay mutations and relate them to specific pathologies. For a given panel, NGS makes it possible to test all sequences in parallel without substantially increasing the cost.

This is leading to a new clinical practice in which all known contributing genes of a pathology can be assessed at first evaluation. However, the results of such an analysis may be quite complex, involving up to thousands of variant calls that should be sorted and filtered with great care to arrive at results with clinical significance.

In this case study, we illustrate how a French pediatric hospital, Robert-Debré, has implemented Alissa Interpret, the variant assessment and reporting module on the Agilent Alissa Clinical Informatics platform, to automate and manage variant assessment in their NGS workflow.

NGS activities at Robert-Debré Hospital

Robert-Debré is a university hospital located in Paris. As one of France's premier hospitals for children and expecting mothers, it is reputed for its pediatric research. Robert-Debré is also an official reference center for a number of pathologies, including developmental diseases and malformative syndromes.

For three of these, congenital microcephaly, RASopathy, and leukodystrophy, the hospital's molecular lab has developed gene panels. In 2015, the lab processed 500 index cases using NGS for these three disorders. As NGS is now being integrated in first-line diagnosis, this number will likely increase.

At Robert-Debré, the Alissa Interpret software platform is used for sorting, filtering, annotating and classifying variants. Variants classified as Class 3 to 5 (e.g. VOUS, likely pathogenic, pathogenic) are subsequently verified with Sanger sequencing before the lab issues a final report. The annotated results are further used to enrich the lab's variant database.

Conclusion Summary

Alissa Interpret offers an accessible, intuitive, and time-saving way to set up a classification strategy to manage the extensive sets of variants coming out of NGS sequencing for the molecular geneticists at Robert-Debré.

The microcephaly gene panel

Robert-Debré has implemented Alissa Interpret in their NGS pipeline as a way to triage variants more efficiently. To do so, they made use of the software's extensive options to configure dedicated filtering and classification trees for each of the three focus pathologies. Here, we illustrate the filtering strategy adopted for microcephaly. At 200 kilobases, the microcephaly gene panel covers 39 genes involved in isolated microcephaly, primitive microcephaly, or in microcephaly associated with significant growth retardation, such as Seckel syndrome or Meier-Gorlin syndrome.

Microcephalies form a group of rare diseases with a high genetic heterogeneity. Therefore the detected mutations are private mutations. In addition, the genes involved show numerous polymorphisms. So the genetic picture is quite heterogeneous, which complicates managing and sorting variants and illustrates the advantage of a dedicated tool.

Robert-Debré's variant classification strategy for microcephaly

The molecular diagnostics lab at the Robert-Debré hospital has set up a classification strategy that tackles a number of hurdles at once. It guarantees no sample swap has taken place, identifies variants that have been described before, investigates the impact of variants at the protein level and excludes variants common in the population, including the hospital's own microcephaly patient cohort. We will describe how the lab has built an efficient labeling and variant review strategy for microcephaly, a strategy that also enabled Robert-Debré to strengthen their own knowledge base via the Alissa Interpret Managed Variant List (MVL) functionality. **Figure 1** below gives an overview of the full classification tree.

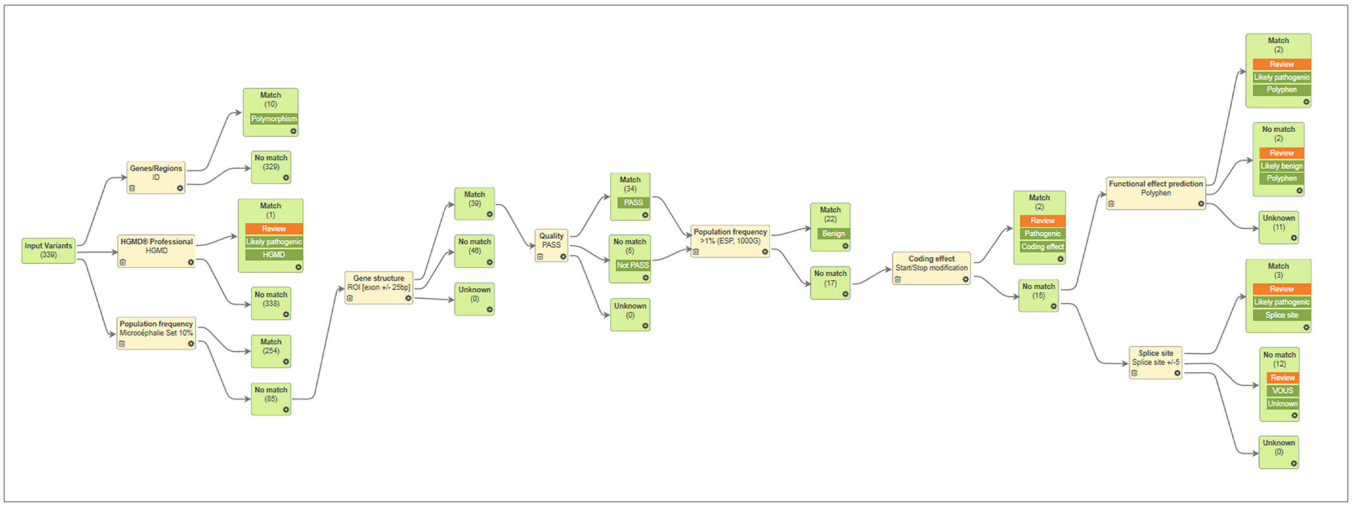

Figure 1. Robert-Debré's classification tree for microcephaly.

Variant assessment via a classification tree

Addressing specific needs via flexible labeling and filtration

As a first step to sort the 339 variants in this example, three filters are defined. The lab has used the Genes/Region filter to ensure no sample inversion took place during the preparation of libraries. This was done by filtering the NGS-sequenced genome against the results from an 11-loci SNaPshot assay. After this initial check, the variants are filtered for their presence in the HGMD Pro database. As defined by the lab, these variants will receive the labels 'Likely Pathogenic' plus 'HGMD' and will be marked for review (red label). The latter enables the lab to manually review these variants in the variant review tab. Given the expectation for rare mutations in microcephaly, the variants are then passed through a population frequency filter.

This filter is shown in **Figure 2**, on the right, highlighting the reference set functionality. Here, a reference set was compiled for a cohort of 55 in-house microcephaly patients. In this way, the lab was able to specify the desired cut-offs for minimal allele count and frequency, to exclude common variants based on their own patient population.

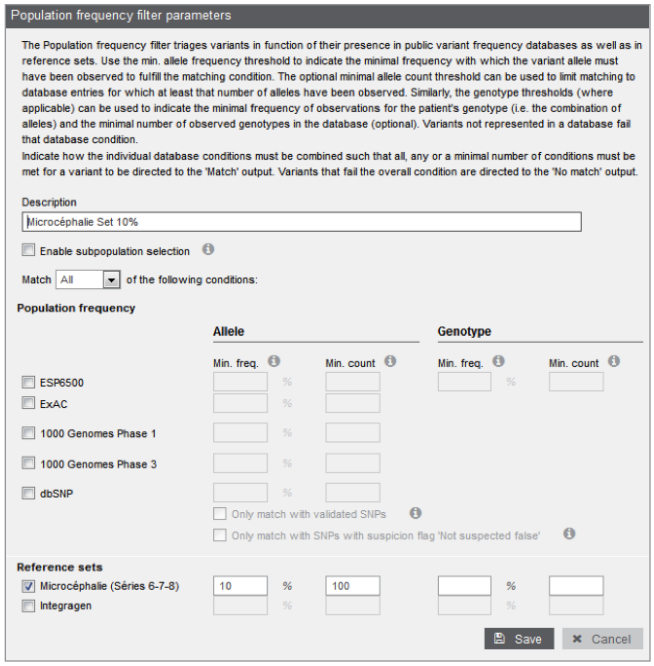

Figure 2. Population frequency filter, strengthened with a reference set compiled for a cohort of 55 in-house microcephaly patients, to exclude variants that are common in the patient population.

Identifying false negative artifacts

After the application of a Gene structure filter to focus on the exonic (±25bp flanking intronic) regions, the lab came up with an inventive way of using Alissa Interpret's labeling functionality to identify potential false negative artifacts, see **Figure 3**, below.

Usually, by applying a quality filter to retain the variants with the PASS status, most excluded variants will be true artifacts. However, some of these might be valid variants annotated with low frequency or low read depth, which could be due to mosaicism or shallow coverage. By making use of the flexible labeling, the lab guarantees to keep track of all variants.

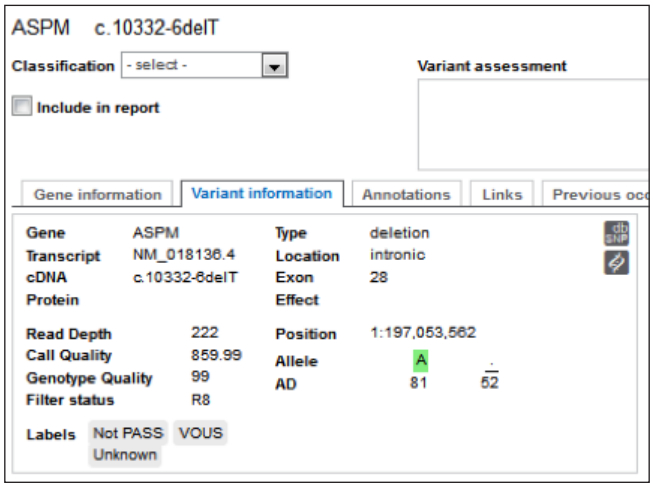

Figure 3. Artifactual variant flag as "Not PASS." This variant is a deletion and the number of adjacent repeats in the reference is greater than 8, this variant is annotated R8 in filter status on VCF file.

Pre-classification filtering

Following these steps, variants present in the ESP6500 and 1000 Genomes databases with a minimal allele frequency of 1% will receive the label 'Benign.' At this point, only 17 variants out of the 339 are left and these will next be evaluated for coding effects. The variants annotated as a frameshift, stop-gain, stop-loss or start-loss mutation will receive the label 'Pathogenic' and are therefore marked for review. For the other variants, the functional effect will be predicted. To do this, the Polyphen-2 algorithm was chosen from a large number of functional effect prediction algorithms available. All variants going through this filter will be marked for review, but only the ones predicted to be possibly/probably damaging will be labeled as 'Pathogenic.' The variants located within 5 bases of a canonical splice site will be labeled 'Likely Pathogenic,' the other ones will receive a 'VOUS' label. Following this pre-classification filtering, the number of variants for review has been effectively lowered to a manageable number that are now ready for manual inspection in the variant review tab.

Manual review and classification of variants in the variant review section

In the variant review tab, annotation information can be consulted to support variant interpretation. In **Figure 4** below, the tab displays annotation ontology (a.o.) information on variant frequencies coming from population databases as well as information from the lab's internal patient population. Moreover, previous occurrences can be examined, and in the variant assessment box, there is room to add expert information concerning this particular variant. All this information can be taken into account to validate the classification of the variant.

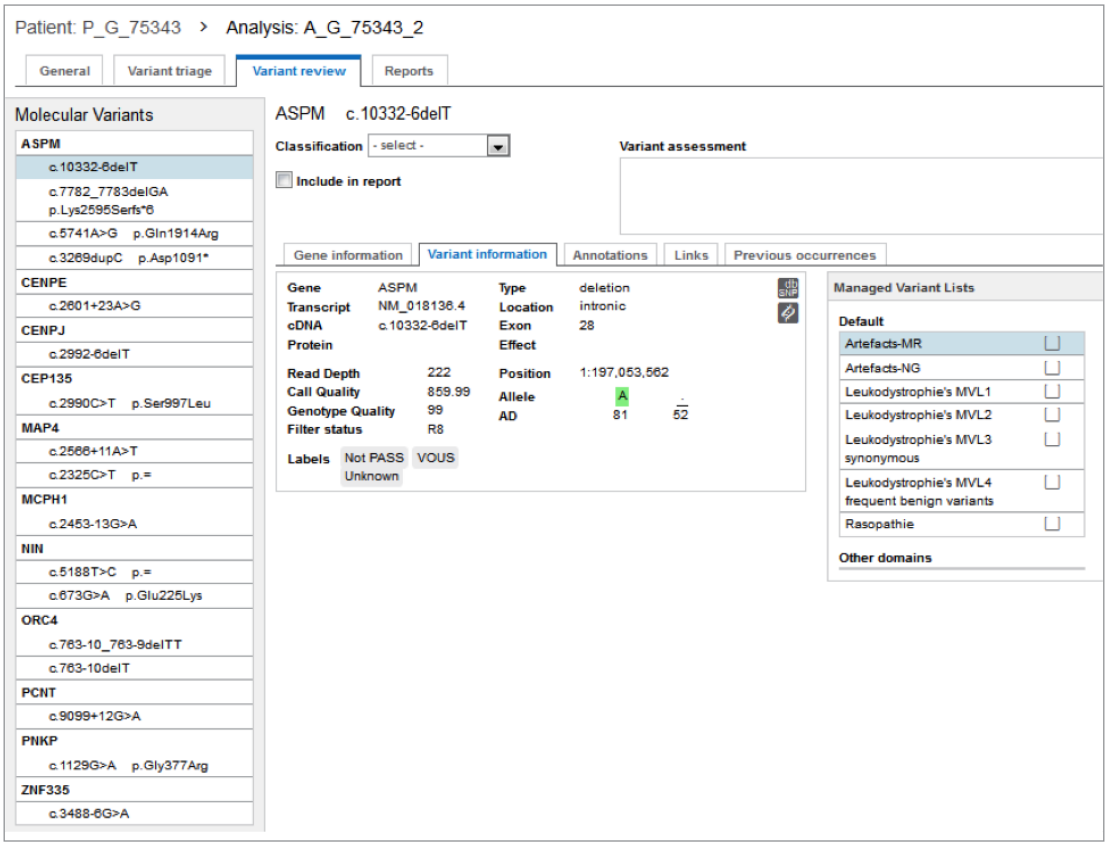

Figure 4. The variant review section collects all the information sources needed to support the variant classification.

Management of internal knowledge: the Managed Variants Lists (MVLs)

After validation of the variant's classification, variants can be added to a managed variant list (MVL). The MVL functionality enables the lab to build its own internal knowledge base that can be used to manage known disease-associated variants but also artifacts. Figure 6 below shows an example of Robert-Debré's MVL for RASopathies.

At Robert-Debré, variants identified by Sanger sequencing before the use of NGS have been added to the MVL. So when they come across a new variant that had been classified as pathogenic before, its classification will take less time. Moreover, the MVL-entries can easily be updated to add new or updated information.

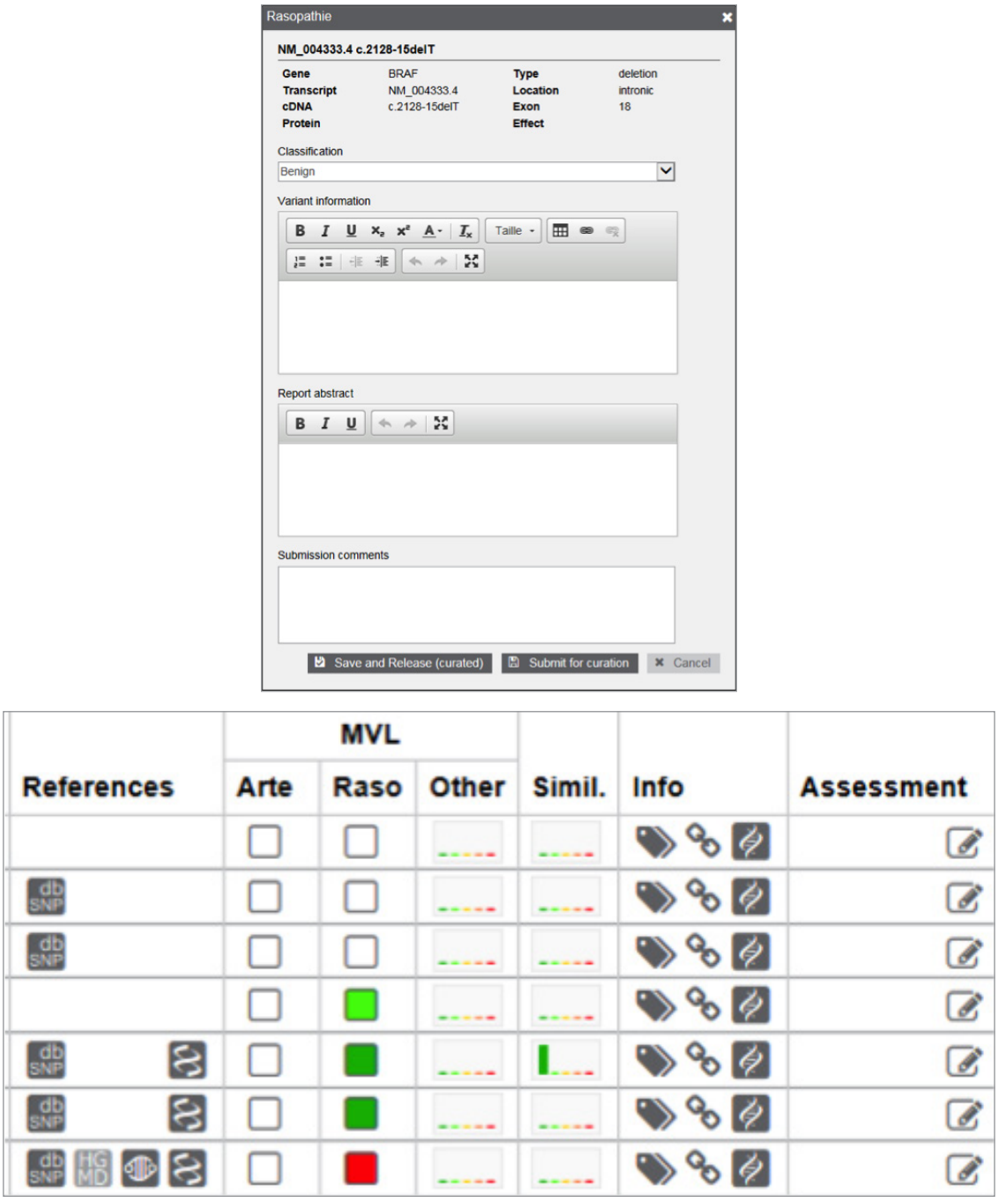

Figure 6. The Managed Variant List (MVL) is the lab's internal knowledge base; it can be updated at any time to add new insights.

Conclusion

For the molecular geneticists at Robert-Debré, Alissa Interpret, the variant assessment and reporting module on the Agilent Alissa Clinical Informatics platform, offers an accessible, intuitive and time-saving way to set up a classification strategy to manage the extensive sets of variants coming out of NGS sequencing.

Robert-Debré successfully used the flexible labeling system to meet its specific needs, also benefiting from the wide variety of data sources available. Moreover, the lab was able to strengthen its workflow with its patient population data via the reference set functionality and via its variant database or MVL. This MVL was further enriched with variants identified by Sanger sequencing, resulting in what they call 'the memory of the lab'.

Outlook

As for the future, Robert-Debré looks forward to sharing its expertise and variant data, pointing as an example to the AchroPuce consortium, a data-sharing network supported by Alissa Interpret.

An efficient clinical pipeline for microcephaly, RASopathy and leukodystrophy gene panels

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.

Trusted Answers. Together.

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