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Case Study:

Whole-Exome Sequencing Diagnostics For Patients with Intellectual Disability at UMC Utrecht: a Tiered and Automated Approach Using Alissa Interpret

At a Glance

In this case study, you will learn:

- How the UMC Utrecht has implemented an automated and tiered approach for WES diagnostics using Alissa Interpret.
- How UMC Utrecht's tiered analysis workflow has been used for a specific clinical case.
- How the tiered approach maximizes clinical utility and time efficiency, while minimizing uncertain and unsolicited findings.

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Introduction

Diagnostic trio Whole-Exome Sequencing (WES) has proven to be an important tool for diagnosing heterogeneous genetic diseases. Especially for patients with syndromic or non-syndromic intellectual disability, WES is increasingly being used as part of the genetic diagnostic workup.

In order to provide the best standard of service to its referring physicians and their patients, the Genome Diagnostics laboratory at the University Medical Center Utrecht (UMC Utrecht) has set-up its ISO15189:2012 accredited exome analysis and reporting pipeline in accordance with the recommendations of the European Society of Human Genetics and the Health Council of the Netherlands.

Key aspects of these recommendations that have been implemented include:

- Informed consent procedure with focus on patient autonomy
- Protocolled procedure for reporting of unsolicited findings
- International collaboration and data sharing to facilitate the interpretation of genomic data

Alissa Interpret, the variant assessment and reporting automation module of Agilent Alissa Clinical Informatics platform, supports variant filtration and interpretation and plays a central role in enabling the implementation of these guidelines via a classification strategy that maximizes clinical utility and minimizes unsolicited findings.

In this case study, the implementation of UMC Utrecht's tiered workflow will be demonstrated via a clinical use case. Likewise an overview of the results achieved by implementing this tiered workflow will be given.

> "The tiered and automated analysis approach has enabled us to limit analysis times between 5 and 30 minutes per case, maximizing clinical utility and time efficiency at the UMC Utrecht." Koen van Gassen, PhD, University Medical Center Utrecht

Conclusion Summary

Through our standardized approach, we have been able to bring the number of unsolicited findings and human error to a minimum. The implementation of this tiered workflow in Alissa Interpret has proven to be an efficient and scalable solution for high-throughput WES diagnostics.

Tiered analysis approach in Alissa Interpret

In order to obtain a quick, reproducible, and largely automated WES analysis workflow, the UMC Utrecht has developed a tiered analysis set-up:

- Tier 1 Gene Panel Analysis
- Tier 2 De Novo Exome Analysis
- Tier 3 Recessive Exome Analysis

Each tier has its own dedicated filtration tree that is optimized for its purpose and reporting guidelines. As shown in **Figure 1,** the tiered workflow continues until a diagnosis is obtained, which limits analysis times to between five and 30 minutes.

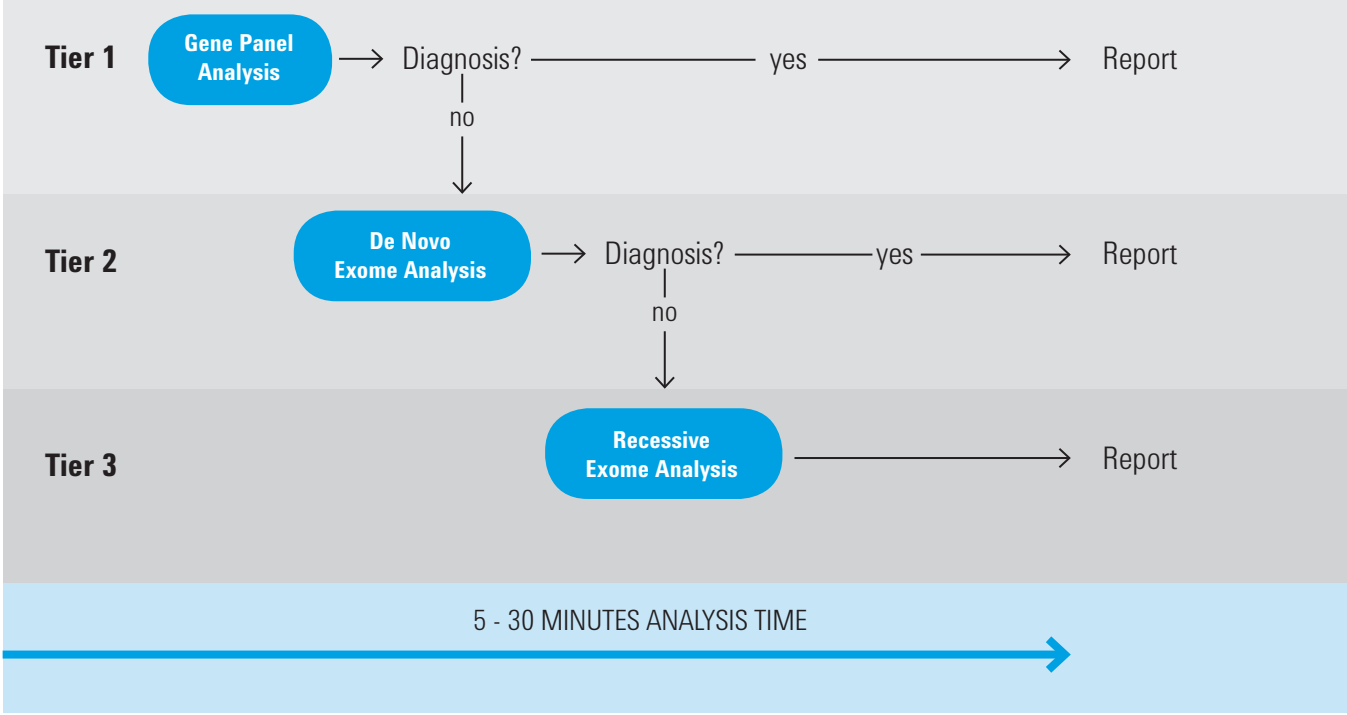

Figure 1. Workflow overview of tiered analysis approach developed at UMC Utrecht and implemented with Alissa Intepret. By implementing this workflow, the lab was able to reduce analysis times to between five and 30 minutes.

Illustration of the tiered workflow through a clinical use case

A 5-year-old male child affected with seizures, developmental delay, hypotonia, spasticity, sensorineural hearing loss and gastrointestinal issues was referred for trio WES. As previous extensive genetic and metabolic diagnostics had not resulted in a diagnosis, the case was subjected to the tiered approach.

Tier 1 resulted in the identification of a heterozygous pathogenic mutation in the PEX1 gene, known to be associated with the autosomal recessive Zellweger syndrome. But clinical features did not match this condition and a second mutation was not detected. Therefore, the analysis was continued in tier 2.

Tier 2 resulted in the identification of a de novo heterozygous variant of uncertain clinical significance (VUS) in the TNRC18 gene. This gene has not previously been associated with clinical features and was therefore considered to be a candidate gene. Since this finding did not result in a diagnosis, analysis continued to tier 3.

Tier 3 resulted in the identification of two compound heterozygous mutations in the SPATA5 gene: one nonsense and one missense mutation. Submission of this gene to the data sharing database of GeneMatcher (http://www.genematcher.org) resulted in several matches. By comparing genotypes and phenotypes between several laboratories the SPATA5 gene was proven to be a causative factor for the observed phenotype (The American Journal of Human Genetics. 2015 Sep 3;97(3):457-64).

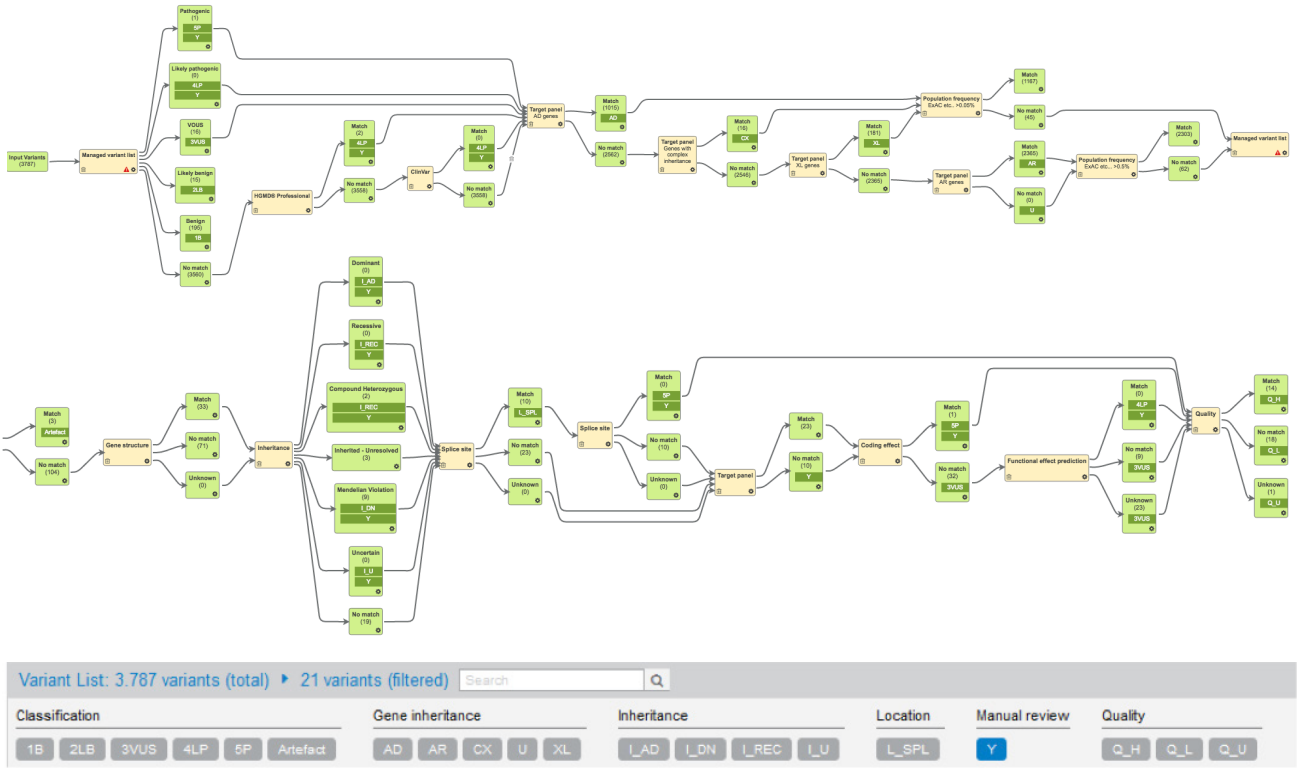

Figure 2. Classification tree of tier 1 (Gene Panel), split in two parts for readability.

Classification Strategies

Tier 1 classification strategy

We have set up a classification tree based mainly upon population frequencies and our internal variant knowledge base or the so-called Managed Variant List (MVL). The label 'manual review : Y' is used for one-click filtering of variants. In this first tier, 21 out of the 3787 variants were left for manual curation.

Tier 2 classification strategy

Shown in **figure 3** is the de novo filtering, based upon population frequencies (including in-house population of healthy parents) and variants identified in the parents. Here, the variants to be reviewed manually are again indicated by the 'manual review : Y'-label. In the end, 21 variants out of 88, 486 variants were left for manual curation.

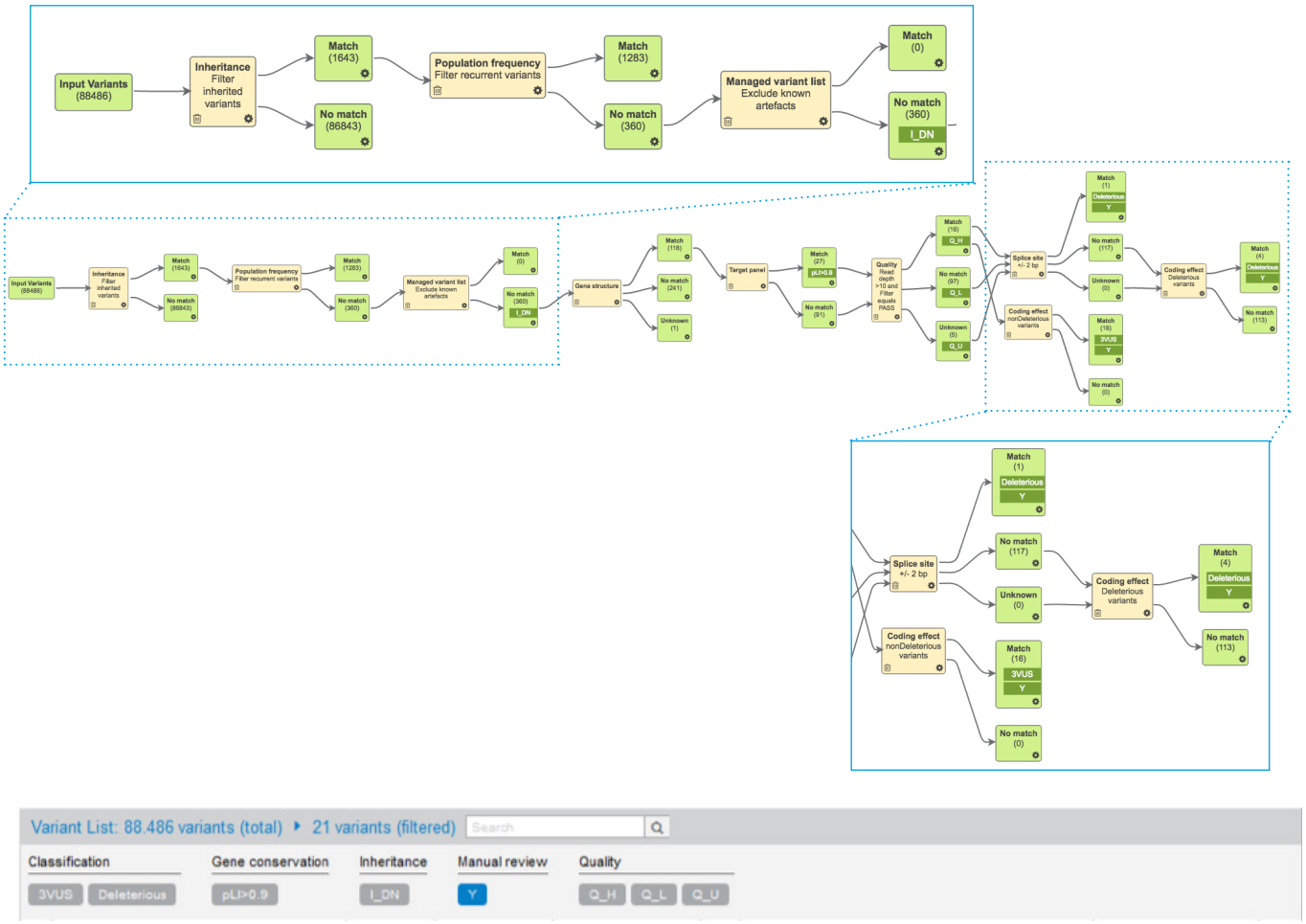

Figure 3. Classification tree of tier 2 (De Novo Exome Analysis).

Classification strategy for Tier 3

In **Figure 4**, the classification tree for the recessive analysis part is shown. In this third tier, filtering is based upon population frequencies, coding effect and recessive inheritance models (homozygous, hemizygous and compound heterozygous). The label 'manual review : Y' is used as before, and in this case only 7 variants out of the 88 486 were left for manual review. In blue, the filter path for one of the compound heterozygous mutations in the SPATA5 gene is highlighted. And as mentioned before, out of these 7 variants two compound heterozygous mutations in the SPATA5 gene could be identified as causal variants (see **Figure 5**).

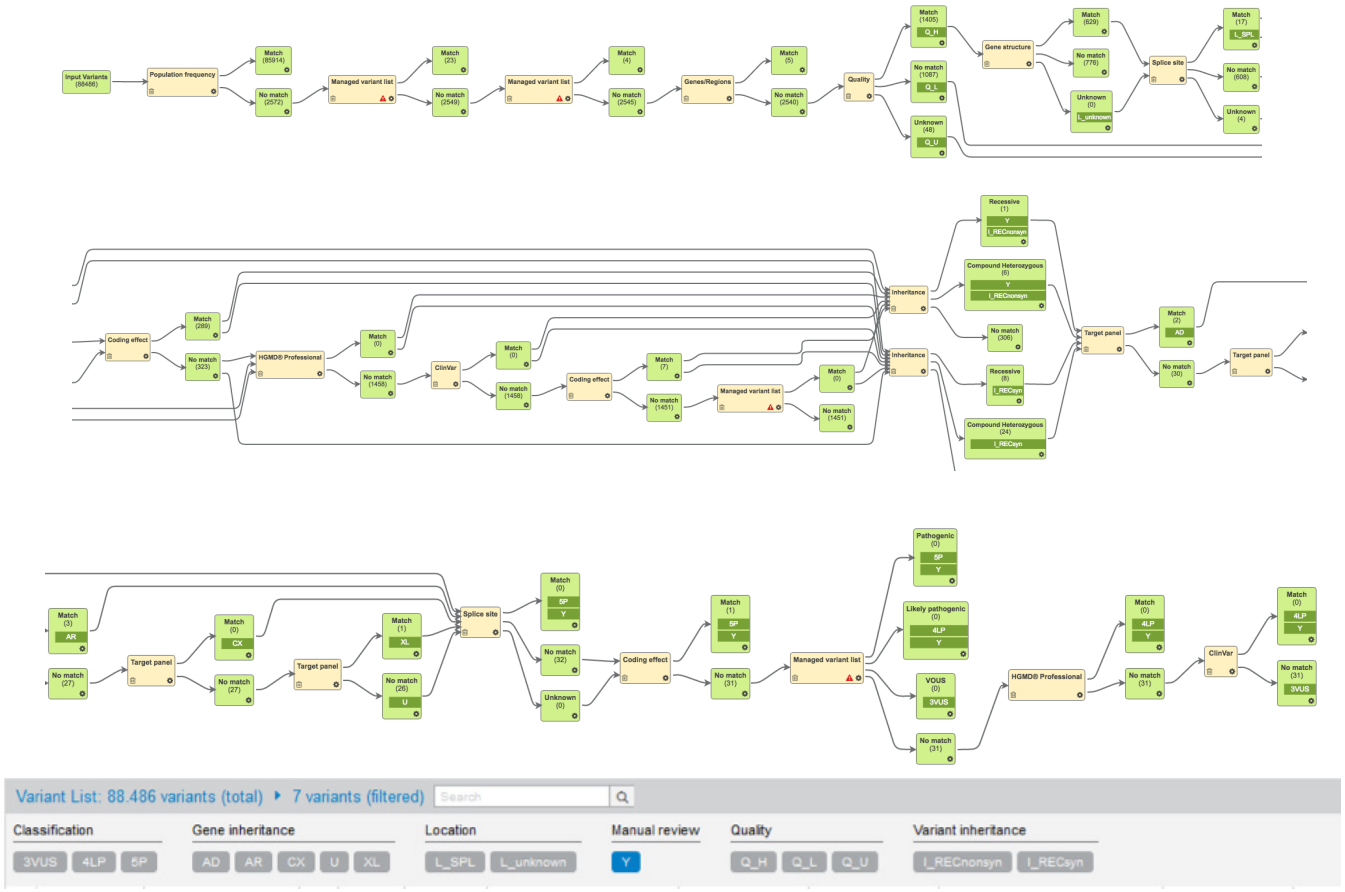

Figure 4. Classification tree of tier 3 (Recessive Exome Analysis) split in tree parts for readability.

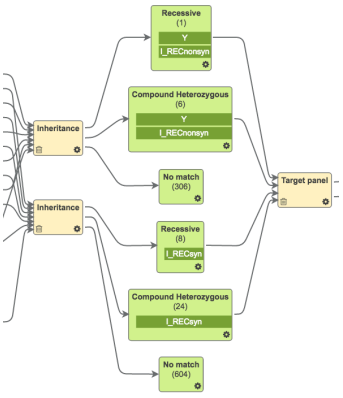

Figure 5. Detail of compound filtering in tier 3.

Results of the tiered approach at University Medical Center Utrecht

Via the tiered and automated approach we presented, UMC Utrecht achieved a substantial gain in clinical utility of its analysis and interpretation resources. As shown in **Figure 6**, 47% of patients was diagnosed through the tiered approach. For an additional 34% of the patients, candidate variants could be identified. Overall the number of variants left for manual curation in each tier did not exceed 20 variants, with most of the times only a handful of variants left to review. The amount of unsolicited findings was minimized to less than 3% and the combination of this tiered analysis workflow and data sharing resulted in numerous publications (see references).

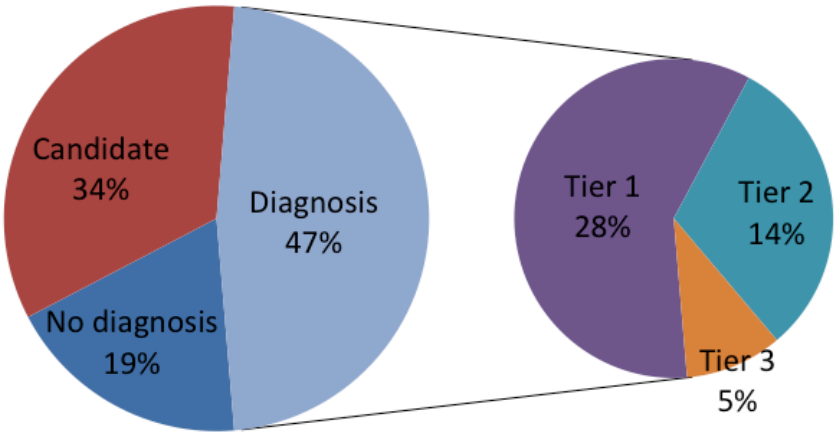

Figure 6 (right). 47% of the patients received a diagnosis through the tiered approach. More than half of them were diagnosed after analysis in the first tier. Moreover, for an additional 34% of the patients 'candidate variants' could be identified through the same analysis approach.

Conclusions

By applying this tiered analysis workflow, the manual decision-making has been limited and analysis times were reduced to between 5 and 30 minutes. Moreover through our standardized approach, we have been able to bring the number of unsolicited findings and human error to a minimum. Accordingly, the diagnostic pipeline has been optimized through the combination of high quality WES, sensitive data analysis and international data sharing. In conclusion, the implementation of this tiered workflow in Alissa Interpret has proven to be an efficient and scalable solution for high-throughput WES diagnostics.

References of publications that include patients diagnosed by using this tiered analysis workflow

1. Human Genetics. 2015 Jan;134(1):97-109
2. The Journal of Clinical Investigation. 2015 Aug 3;125(8):3051-62
3. The Am. Journal of Human Genetics. 2015 Aug 6;97(2):343-52
4. The Am. Journal of Human Genetics. 2015 Sep 3;97(3):457-64
5. The Am. Journal of Human Genetics. 2015 Sep 3;97(3):493-500
6. Genetics in Medicine. 2016 Feb 4
7. JIMD Reports. 2016 Feb 27
8. Neurogenetics. 2016 Mar 22

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.

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