Whole-Exome Sequencing diagnostics for patients with intellectual disability at UMC Utrecht: a tiered and automated approach using Cartagenia Bench Lab NGS

Authors: Koen Van Gassen¹, PhD ; Prof. Hans Kristian Ploos van Amstel¹, PhD
Steven W. Van Vooren², PhD | Tina Smets³, Msc

1. Department of Genetics, University Medical Center Utrecht, The Netherlands
2. Cartagenia, Inc (a part of Agilent Technologies), Boston, MA, USA
3. Cartagenia (a part of Agilent Technologies), Leuven, Belgium

At a glance

In this case study, you will learn:

1. How the UMC Utrecht has implemented an automated and tiered approach for WES diagnostics using Cartagenia Bench Lab NGS.

2. How UMC Utrecht’s tiered analysis workflow has been used for a specific clinical case.

3. How the tiered approach maximizes clinical utility and time efficiency, while minimizing uncertain and unsolicited findings.

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.

“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
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

The Cartagenia Bench Lab NGS platform used for variant filtration and interpretation, plays a central role to enable 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.

**Tiered analysis approach in Cartagenia Bench Lab NGS**

In order to obtain a quick, reproducible and largely automated WES analysis workflow, the UMC Utrecht has developed a tiered analysis set-up. Each tier has its own dedicated filtration tree, optimized for its purpose and reporting guidelines. As shown in figure 1, the tiered workflow continues until a diagnosis is obtained, limiting analysis times to between 5 and 30 minutes.

![Diagram of tiered analysis approach](image)

**Figure 1:** Workflow overview of tiered analysis approach developed at UMC Utrecht and implemented in Cartagenia Bench Lab NGS. By implementing this workflow, the lab was able to reduce analysis times to between 5 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 strategy for Tier 1

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.

Classification strategy for Tier 2

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 \textit{SPATA5} gene is highlighted. And as mentioned before, out of these 7 variants two compound heterozygous mutations in the \textit{SPATA5} gene could be identified as causal variants (see figure 5).

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).

**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 Cartagenia Bench Lab NGS 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**

6. Genetics in Medicine. 2016 Feb 4
7. JIMD Reports. 2016 Feb 27