The Future of **Next-Generation Sequencing** in **Pediatric Neurology;** Trends and Projections

by Mohammad-Reza Ghasemi

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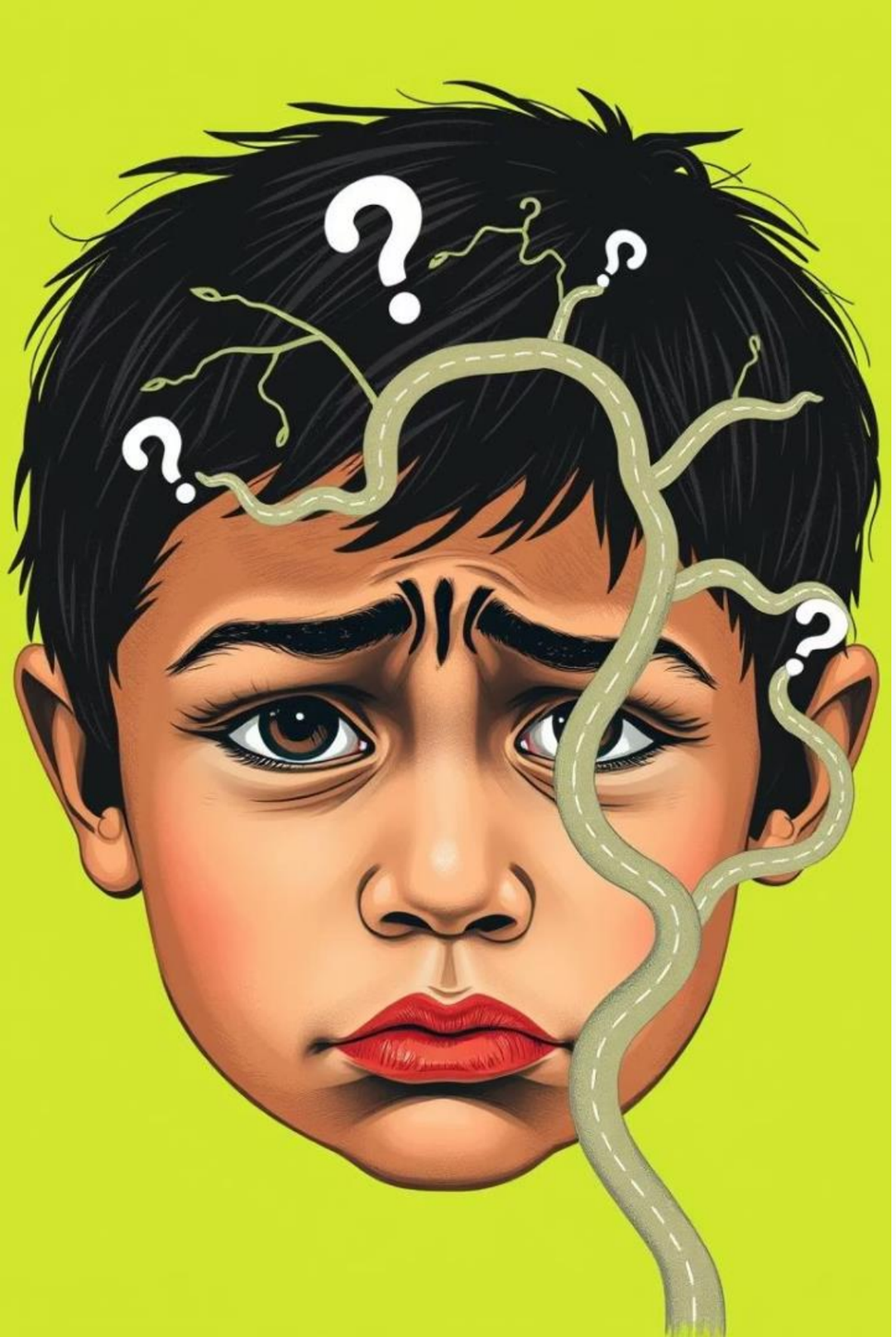
Medical Genetic Lab director



Introduction

- **Rare diseases**: impact small populations, no universal definition (EU: ≤5/10,000)
- Over 7,000 rare diseases identified; affect 30M in US, 29M in EU, **~400M worldwide**
- ***80% genetic; 70% manifest in childhood; ~3% neonatal***
- ***95% lack approved treatments***; mainly symptomatic care
- ***Approximately 80% of genes*** have an active expression in the **brain**.
- **40%** of all known genetic disorders affect **the central nervous system**.
- Average diagnosis time: **4-8 years**; 30% children die before age 5
- Challenges: access, high costs, psychological stress, diagnostic delays

1. Marwaha S, Knowles JW, Ashley EA. A guide for the diagnosis of rare and undiagnosed disease: beyond the exome. Genome medicine. 2022 Feb 28;14(1):23.
2. Gürkan H, Satkın NB. The Importance of Genetic Diagnosis in Rare Diseases. Balkan Medical Journal. 2025 Mar 3;42(2):92.

The Diagnostic Odyssey: A Shifting Paradigm

The "diagnostic odyssey" for rare diseases is a well-known challenge for pediatric neurologists and geneticists.

We are witnessing a fundamental shift: from a phenotype-first, often inconclusive pathway, to a **genotype-driven approach** that delivers definitive answers.

This talk explores the clinical utility and future of NGS, focusing on the synergy between deep phenotyping and rigorous variant interpretation.

A Familiar Problem, A New Solution

The Revolution of Next-Generation Sequencing (NGS)

1

Traditional Genetic Testing

Until about a decade ago, genetic testing was expensive and typically limited to a few genes at a time.

2

NGS Revolution

The advent of next-generation sequencing (NGS) technology has dramatically improved the cost, accuracy, and utility of genetic testing, effectively supplanting older technologies.

3

Parallel Analysis

NGS enables the **rapid and cost-effective analysis of hundreds or even thousands of different genomic regions in a single test**.

4

Dramatic Progress

For perspective, the Human Genome Project, completed in 2001 using Sanger sequencing, took 13 years and $2.7 billion. Today, NGS can sequence the same genome in **2 weeks for around $4,000**, highlighting the extraordinary progress in DNA sequencing.

NGS Technologies: A Brief Overview

Targeted Gene Panels

Cost-effective with ultra-deep coverage, ideal as a first-tier test when clinical features strongly suggest a particular diagnosis.

Whole-Exome Sequencing Sequencing (WES)

Captures 1-2% of the genome (coding regions), balancing coverage and cost, making it a workhorse for diagnostic labs.

Whole-Genome Sequencing (WGS)

Sequences the entire genomic DNA, including non-coding regions and structural variants, offering a comprehensive genetic picture despite higher cost.

Targeted Panels vs. Whole Exome Sequencing

Targeted Gene Panel Sequencing

Focuses on pre-specified genes with a well-defined phenotype.

- High coverage depth
- Lower cost
- Fewer incidental findings

Diagnostic yield: 45-70% for pediatric neuromuscular disorders.

Whole Exome Sequencing (WES)

Sequences all protein-coding regions (2% of genome).

- Ideal for diverse phenotypes
- 25-40% diagnostic yield in undiagnosed cases
- Up to 53% in epilepsy with developmental delay

Trio WES reduces candidates tenfold.

Whole Genome Sequencing: The The Most Comprehensive Approach Approach

Complete Coverage

Covers entire human genome (3 billion base pairs). Provides uniform depth coverage across all regions.

Enhanced Detection

Identifies structural variants, tandem repeats, and intronic variants missed by WES.

High Diagnostic Yield

21-50% in developmental delay and epilepsy cohorts.

Challenges

Complex analysis of ~400,000 variants. Higher cost than other methods.

Article | Open access | Published: 11 July 2024

De novo variants in the *RNU4-2* snRNA cause a frequent neurodevelopmental syndrome

Yuyang Chen, Ruebena Dawes, Hyung Chul Kim, Alicia Ljungdahl, Sarah L. Stenton, Susan Walker, Jenny Lord, Gabrielle Lemire, Alexandra C. Martin-Geary, Vijay S. Ganesh, Jialan Ma, Jamie M. Ellingford, Erwan Delage, Elston N. D'Souza, Shan Dong, David R. Adams, Kirsten Allan, Madhura Bakshi, Erin E. Baldwin, Seth I. Berger, Jonathan A. Bernstein, Ishita Bhatnagar, Ed Blair, Natasha J. Brown, ... Nicola Whiffin

+ Show authors

Nature **632**, 832–840 (2024) | Cite this article

Abstract

itation

und 60% of individuals with neurodevelopmental disorders (NDD) remain undiagnosed after comprehensive genetic testing, primarily of protein-coding genes[1]. Large genome-sequenced cohorts are improving our ability to discover new diagnoses in the non-coding

The VUS: A Collaborative Investigation

The Variant of Uncertain Significance (VUS) represents a knowledge gap and our primary diagnostic hurdle.

Not a Final Answer

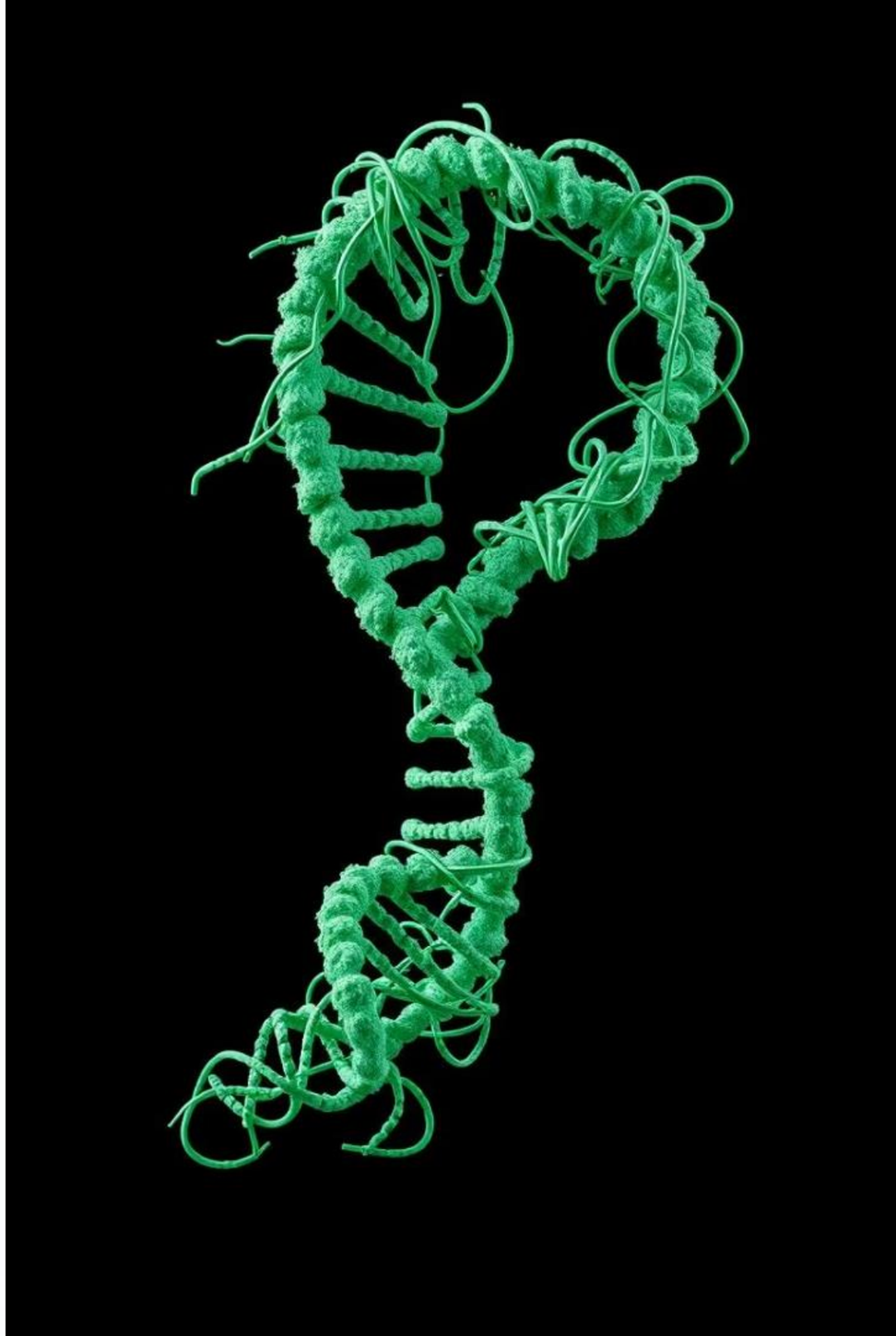
It's the start of a collaborative process.

Neurologist & Geneticist

Deep phenotyping and rigorous interpretation per ACMG/AMP guidelines.

Ongoing Re-evaluation

Up to 20% of VUSs are reclassified over time with new evidence.



All Cases > M.R.GH

M.R.GH

Assay: Germline case from VCF | Assignee: Unassigned | Status: Active

Follow Case

SNP | SV

Case Details

Workbench

Variants

Case History

Search variants

FILTERS 3

- Phenotypes
- Panel
- Gene Properties
- Franklin Classification 3
  - [ ] Pathogenic
  - [ ] Likely Pathogenic
  - [x] Uncertain
    - [x] Leaning Pathogenic
    - [x] Uncertain Significance
    - [x] Leaning Benign
  - [ ] Likely Benign
  - [ ] Benign
- My Organization Classification
- Classification Tags

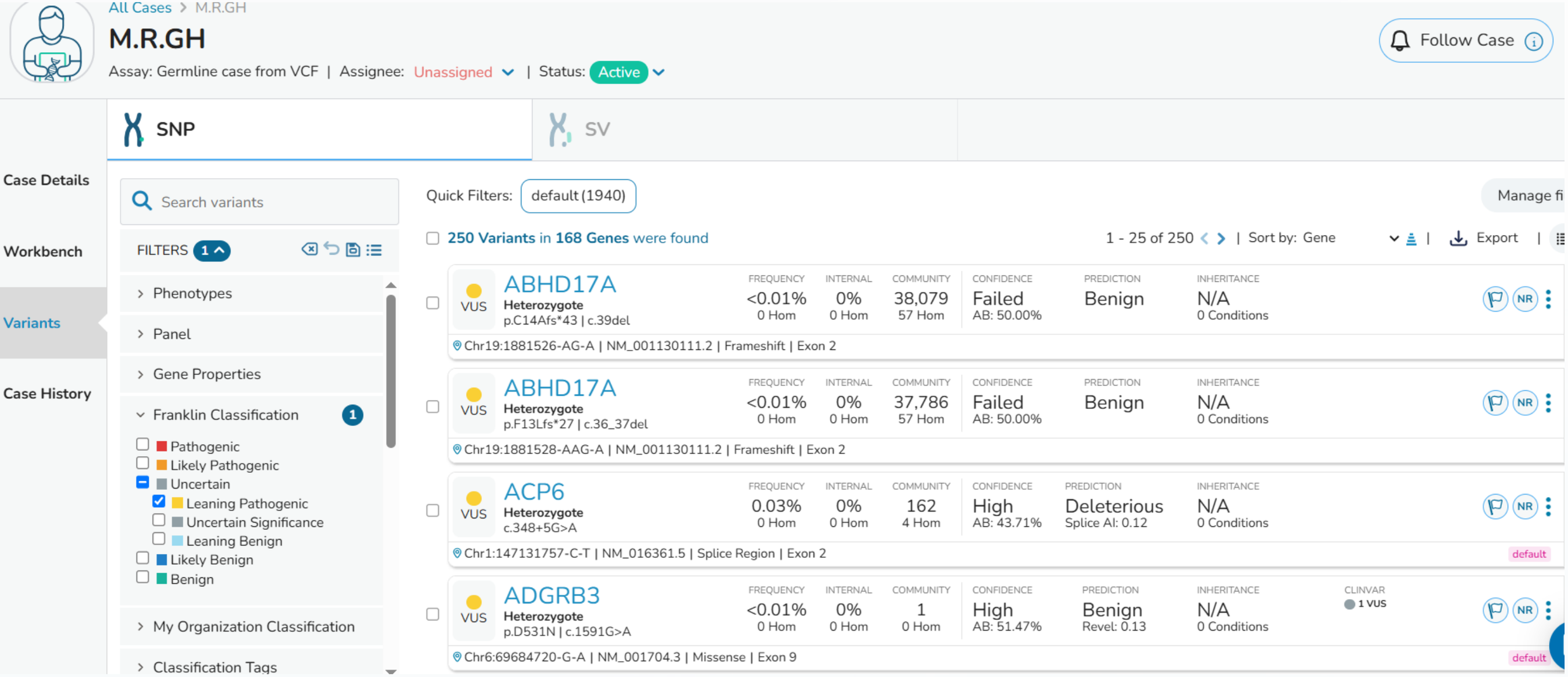
Quick Filters: default (1940)

Manage filters

143,455 Variants in 22,792 Genes were found

1 - 25 of 143,455 | Sort by: Gene | Export

	FREQUENCY	INTERNAL	COMMUNITY	CONFIDENCE	PREDICTION	INHERITANCE
VUS **A1BG** Heterozygote c.1192+252C>A	N/A	0% 0 Hom	N/A	Low AB: 66.67%	Benign Splice AI: 0.02	N/A 0 Conditions
Chr19:58861484-G-T | NM_130786.4 | Regulatory(Intronic) | Exon 6						
VUS **A1CF** Heterozygote c.*8333A>G	N/A	0% 0 Hom	N/A	Low AB: 66.67%	N/A	N/A 0 Conditions
Chr10:52558156-T-C | NM_014576.4 | Downstream | Exon 13						
VUS **A1CF<>PRKG1** Heterozygote n.52692250G>T	N/A	0% 0 Hom	N/A	Low AB: 66.67%	N/A	N/A 0 Conditions
Chr10:52692250-G-T | | Intergenic | Exon						
VUS **A1CF<>PRKG1** Homozygote n.52695237T>C	N/A	0% 0 Hom	N/A	Low AB: 100%	N/A	N/A 0 Conditions
Chr10:52695237-T-C | | Intergenic | Exon						



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Search variants

FILTERS 1

- Region
- Effect
- Regulatory
- Chromosome
- Zygosity
- Allele Balance (VAF)
- Frequency
- Confidence
- Prediction
- Evidence

Quick Filters: default (1940)

Manage filt

3 Variants in 3 Genes were found

1 - 3 of 3 | Sort by: Gene | Export

Appeared in 1 papers

LDLR — P
Heterozygote
p.H485R | c.1454A>G

FREQUENCY	INTERNAL	COMMUNITY	CONFIDENCE	PREDICTION	INHERITANCE	CLINVAR (+1)
N/A	0% 0 Hom	16 2 Hom	High AB: 53.57%	Deleterious Revel: 0.92	AR | AD 5 Conditions	1 LP 1 B

Chr19:11224306-A-G | NM_000527.5 | Missense | Exon 10 — default WB

Marked as **Conflicting** by **community** and appeared in **8** papers

OTOA — P
Heterozygote
p.E787* | c.2359G>T

FREQUENCY	INTERNAL	COMMUNITY	CONFIDENCE	PREDICTION	INHERITANCE	CLINVAR
<0.01% 0 Hom	0% 0 Hom	9,611 0 Hom	Failed AB: 23.16%	N/A	AR 4 Conditions	4 P 1 LP 3 VUS

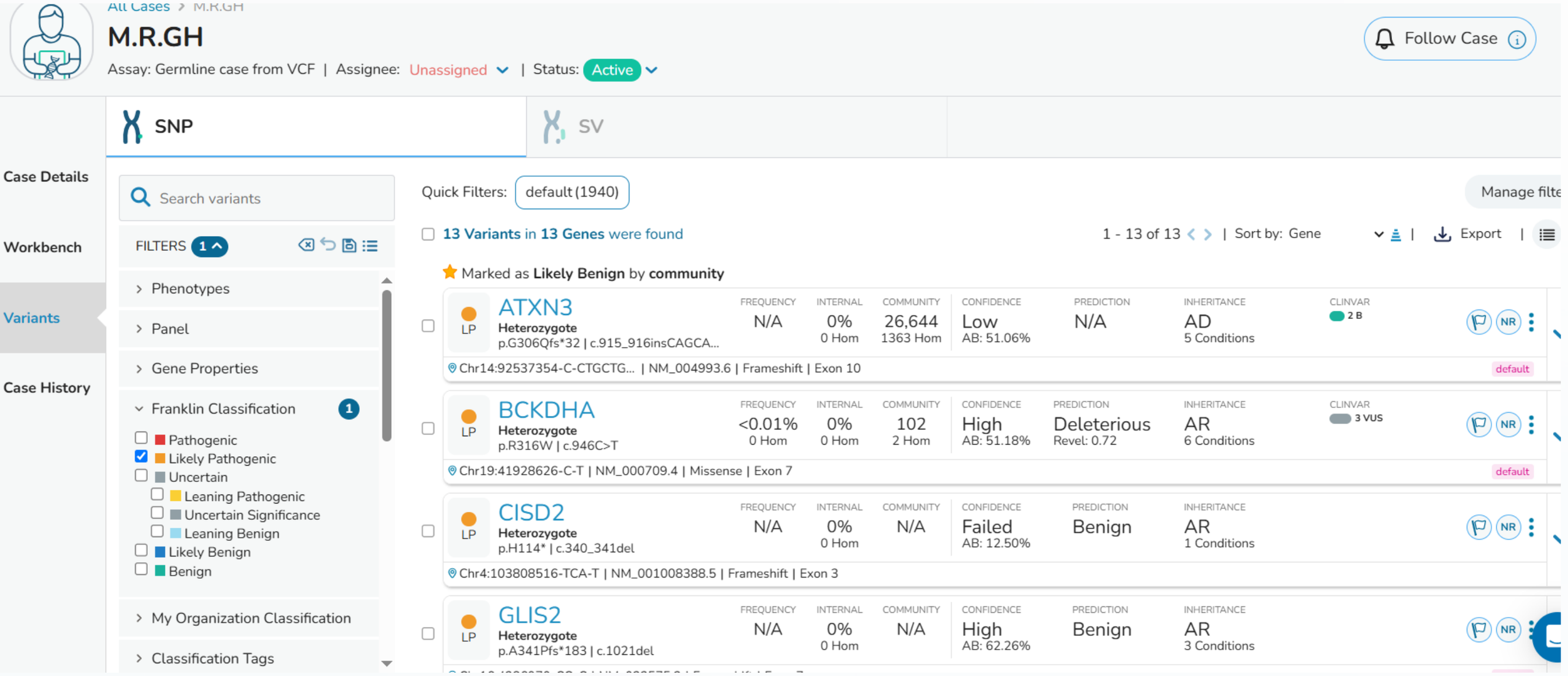
Chr16:21747639-G-T | NM_144672.4 | Stop Gain | Exon 22

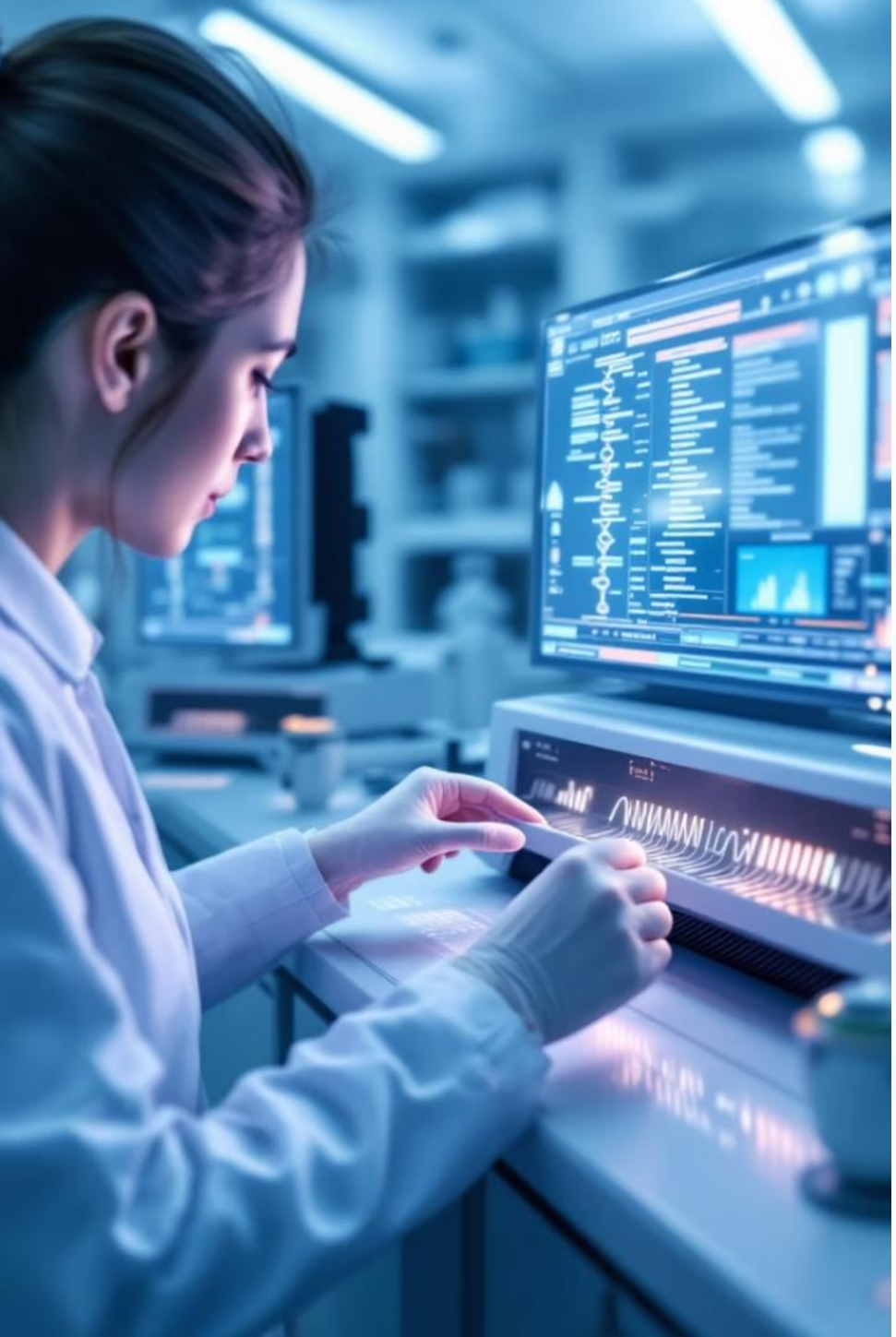
Marked as **Conflicting** by **community** and appeared in **138** papers

PRSS1 — P
Heterozygote
p.N29I | c.86A>T

FREQUENCY	INTERNAL	COMMUNITY	CONFIDENCE	PREDICTION	INHERITANCE	CLINVAR (+1)
<0.01% 0 Hom	100% 0 Hom	109,0... 42 Hom	Failed AB: 17.81%	Uncertain Revel: 0.27	AD 4 Conditions	15 P 2 VUS 1 other

Chr7:142458451-A-T | NM_002769.5 | Missense | Exon 2





Long-Read Sequencing: The Next Frontier

Extended Read Length

Generates 10-60kb reads (up to 2Mb). Dramatically longer than short-read methods.

Improved Mapping

Better alignment in repetitive regions. Detects complex structural variants.

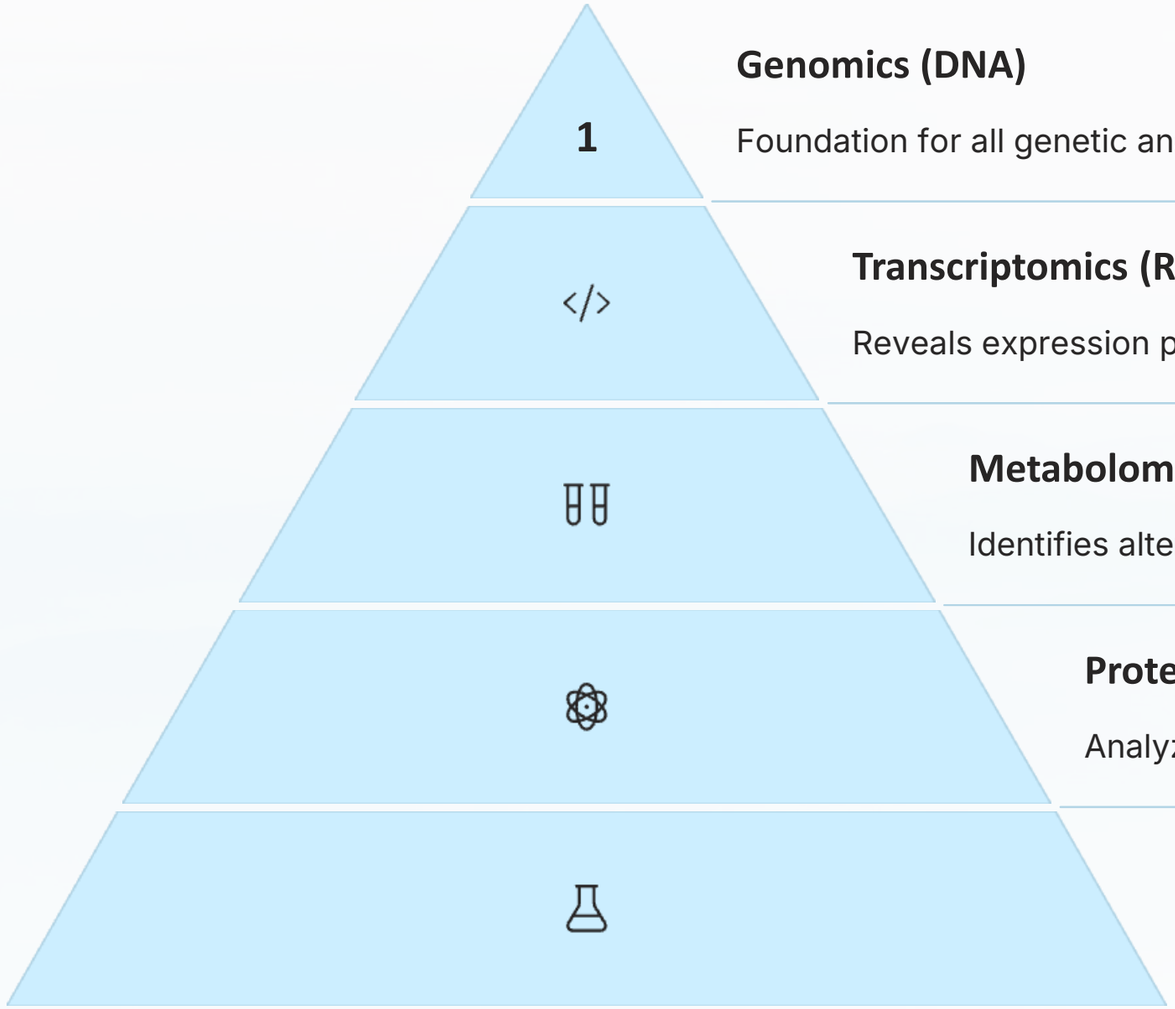
Haplotype Phasing

Assigns variants to parental chromosomes. Identifies compound heterozygous mutations.

Direct Methylation Detection

Analyzes epigenetic markers without conversion steps. Provides comprehensive variant profile.

Beyond Genomics: Multi-omics Approaches



Genomics (DNA)

Foundation for all genetic analysis

Transcriptomics (RNA-seq)

Reveals expression patterns and splicing variants

Metabolomics

Identifies altered metabolites

Proteomics

Analyzes protein expression and modifications

Functional Studies

Validates variants with CRISPR and other methods

Integrating multiple "omic" technologies provides crucial diagnostic insights when genomic sequencing alone is inconclusive.

AI: Finding the Signal in the Noise

Analyzing 3 billion base pairs is a monumental task.

Massive Datasets

Sifts through billions of base pairs.

Variant Prioritization

Prioritizes key genetic changes.

Accelerated Interpretation

Reduces human error, uncovers complex patterns.

Pathogenicity Prediction

Predicts with >95% accuracy.

AI-powered platforms can sift through massive genomic datasets, prioritize variants, and predict pathogenicity with **>95% accuracy**.

Practical Application in Pediatric Neurology: Impact on Patient Care

Enhanced Diagnosis

NGS significantly aids in precisely diagnosing children with epilepsy and/or developmental delay

High Diagnostic Yield

Approximately **24%** for pediatric-onset epilepsies, with higher rates for early-onset conditions

Precision Medicine

Up to **33% of patients** with pathogenic variants become eligible for personalized treatments

Improved Outcomes

Genetic results lead to optimized therapies and better seizure control in many children

Specific phenotypic features associated with a higher diagnostic yield include **epilepsy onset before one year of age, presence of neurological deficits, psychomotor delay/cognitive disability, and malformative aspects on brain MRI**.

One study demonstrated a high diagnostic yield of NGS (**up to 70%**) in children with unexplained epilepsy accompanied by neurodevelopmental delays, particularly for drug-resistant epilepsy. Notably, all five patients with neonatal-onset seizures in this cohort had diagnostic NGS.

Examples of precision medicine include: **sodium channel blockers** for gain-of-function variants in *SCN2A* and *SCN8A*, **mTOR inhibitors** for mTORopathies, and the **ketogenic diet** for Glut1 deficiency syndrome.

In one study, genetic test results led to **optimization of anti-seizure medications or dietary therapies in six children**, resulting in improved seizure control and neurodevelopmental trajectories. For instance, an *SCN1A* mutation prompted a switch from lamotrigine to levetiracetam and ethosuximide, leading to seizure remission.

WES can influence clinical management in **48% of diagnosed patients**, leading to enhanced surveillance, specialist referrals, modifications to diet/lifestyle, and guiding the appropriateness of investigations and medications.

The Local Context: The Challenge with Global Data

Why Global Databases Are Not Enough for Our Patients

Major genomic databases (gnomAD, ClinVar) have a significant **Euro-centric bias**.

Iran's unique genetic landscape, with high rates of consanguinity and specific founder mutations, is poorly represented.

This leads to a high potential for misinterpreting variants—classifying a common local variant as rare and pathogenic, or vice versa.

The National Solution: A Strategic Genome Initiative

A National Leap Forward: Iran's Iran's Strategic Genome Initiative

A landmark initiative, driven by national experts including teams at Sharif University of Technology, is creating a new paradigm for diagnostics in Iran. 110,000 Iranian Genomes Analyzed This project is building a high-resolution genetic map of the Iranian population.

An AI-Powered Analytical Engine

More Than a Database: An Analytical Powerhouse Powerhouse

This platform transcends a simple frequency lookup table; it's an intelligent analytical engine. It is powered by machine by machine learning models trained specifically on 100,000 Iranian genomes, providing accurate, predictive, and discerning and discerning insights.

Data Input

Raw genomic data from Iranian individuals

AI Processing

Machine learning models analyze and interpret variants

Clinical Insight

Population-specific allele frequencies and pathogenicity scores

The system accurately provides population-specific allele frequencies, offers AI-driven pathogenicity scores tailored to our tailored to our genetic background, and differentiates common local polymorphisms from truly rare pathogenic variants. pathogenic variants.

From VUS to a Definitive Diagnosis

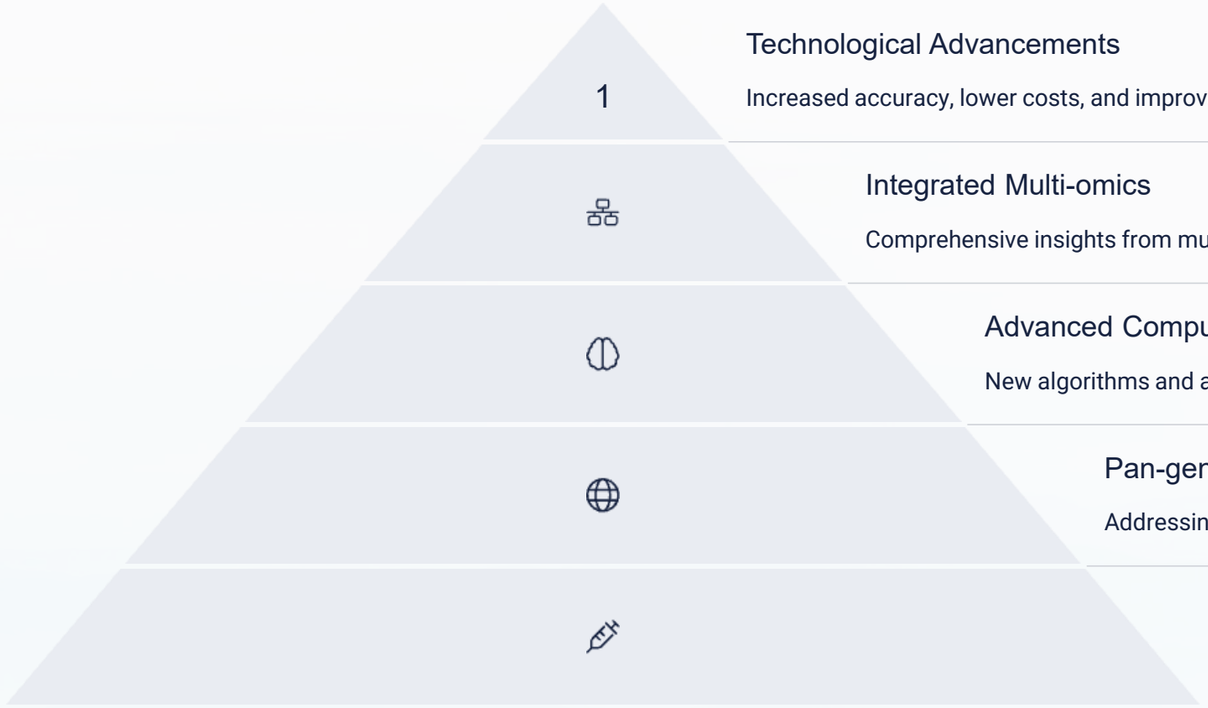
The Clinical Challenge

A child presents with a progressive neurological disorder. Genetic testing reveals a Variant of a Variant of Uncertain Significance (VUS) in a candidate gene. Global databases offer offer ambiguous interpretations, leaving the diagnosis unclear.

The National Platform's Solution

The national platform performs two critical checks. First, a frequency check confirms the confirms the variant's rarity, being absent in 100,000 local controls. Second, its AI model model assigns a high pathogenicity score based on local data. This confidently upgrades the upgrades the VUS to "Likely Pathogenic," leading to a definitive diagnosis and ending the ending the diagnostic odyssey for the family.

Future Trends and Projections in Pediatric Neurogenetics



Technological Advancements

Increased accuracy, lower costs, and improved accessibility

Integrated Multi-omics

Comprehensive insights from multiple biological data types

Advanced Computational Methods

New algorithms and automated analysis approaches

Pan-genome References

Addressing reference and ancestry bias across populations

Precision Medicine Expansion

Gene-targeted therapies transforming previously incurable conditions

Ongoing advancements in sequencing technology and algorithm development are expected to continuously **increase accuracy and lower costs**, making NGS more accessible for routine clinical diagnostics.

Long-read sequencing will further enable the sequencing of challenging genomic regions, leading to the discovery of new associations between genomic regions and genetic disorders.

The integration of big data from **genomics, transcriptomics, proteomics, metabolomics, and epigenomics** promises to provide more comprehensive insights into biological markers, pathophysiology, and genotype-phenotype relationships in complex and undiagnosed diseases.

The development of new **variant prioritization algorithms** and approaches to automate and accelerate genomic analysis will streamline the diagnostic process.

Periodic reanalysis of genomic data, especially for variants of unknown significance (VUS), will become more effective as medical literature and clinical databases evolve.

As genomic investigations increasingly become first-line diagnostic tests in neurology, it is vital for neurologists and epileptologists to be well-equipped to navigate this evolving genetic landscape. General neurologists will remain essential for providing comprehensive, coordinated care in communities, preventing fragmentation of care, and promoting brain health.

Economic Hurdles

Cost Challenges

High data analysis and storage expenses.

Global Disparity

Limited adoption in resource-poor settings.

Funding Needs

Requires equitable models and collaboration.

Ethical Considerations

Incidental Findings

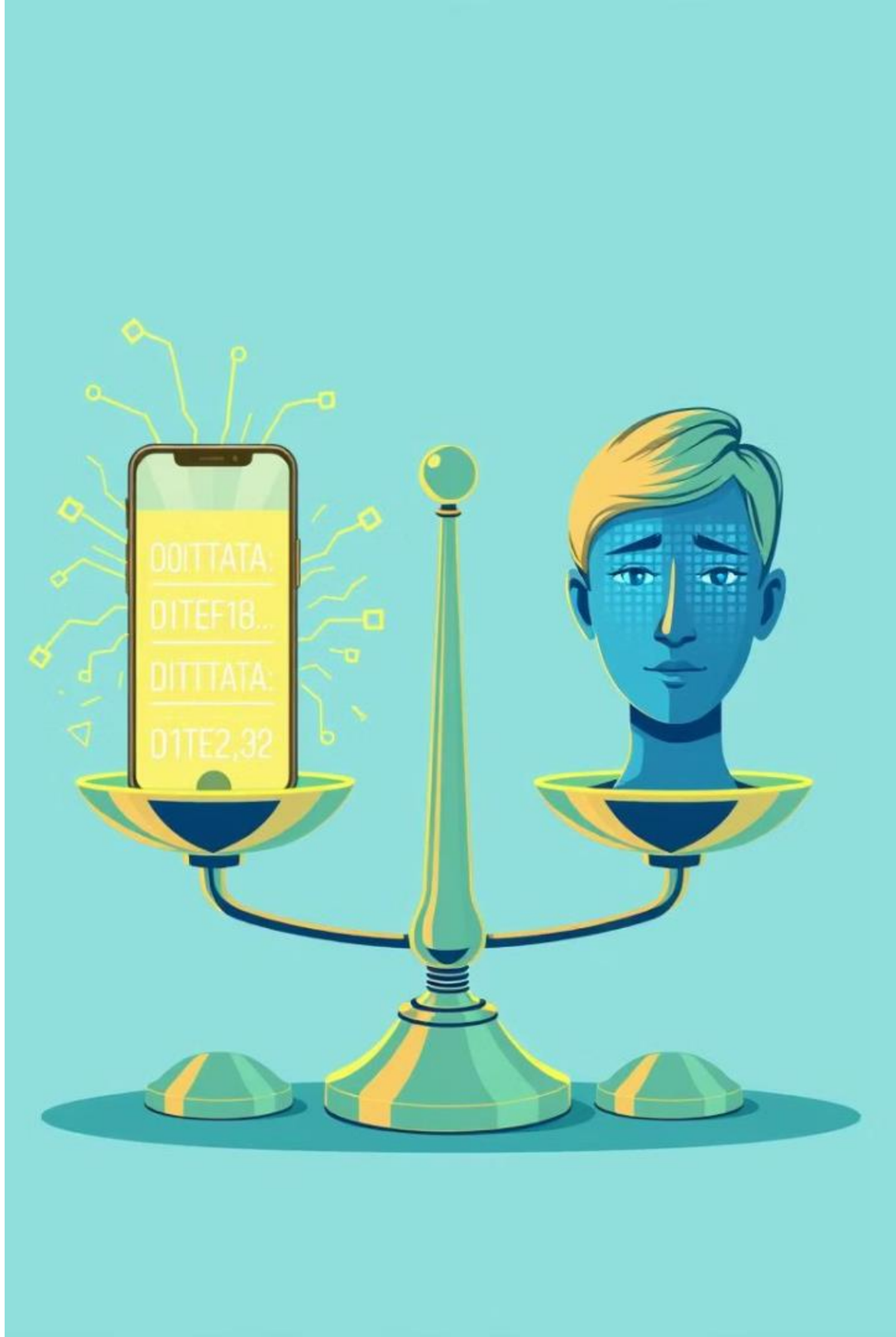
Managing and disclosing unexpected results.

Data Privacy

Ensuring patient information security.

Adult-Onset Conditions

Implications for reporting in children.



Training and Education

Provider Skills

Interpret complex genomic data.

Genomic Integration

Understand implications for patient care.

Continuing Education

Pivotal for NGS potential.

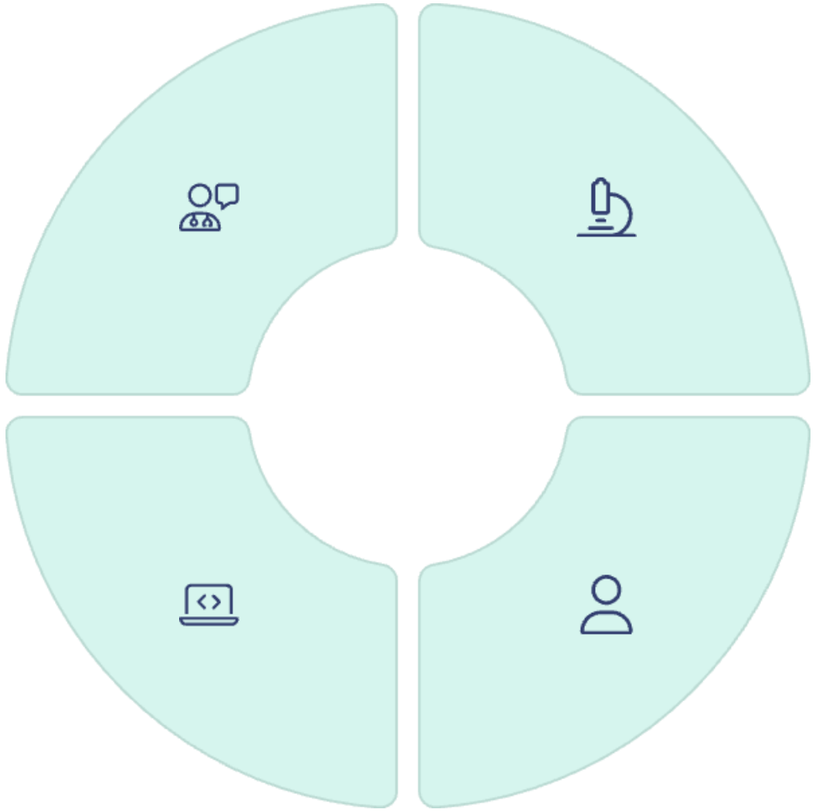
Multidisciplinary Collaboration

Clinicians

Patient care insights.

Medical Geneticist

Molecular interpretation.



Bioinformaticians

Data analysis and tools.

Genetic Counselors

Family support and guidance.

Future Projections

WGS Adoption

Increased use as first-tier test.

Multi-Omics

Integrated data for disease understanding.

Long-Read Sequencing

Accurate structural variant detection.

AI Integration

Streamlined variant interpretation.

Clinical Translation

Earlier Diagnoses

Precise identification of conditions.

Targeted Interventions

Improved disease outcomes.

Personalized Treatment

Refined strategies based on genotype.

NEWS | 15 May 2025

World's first personalized CRISPR therapy given to baby with genetic disease

Treatment seems to have been effective, but it is not clear whether such bespoke therapies can be widely applied.

By Heidi Ledford

A baby boy with a devastating genetic disease is thriving after becoming the first known person to receive a bespoke, CRISPR therapy-for-one, designed to correct his specific disease-causing mutation[1].

Conclusion: The New Era of Precision Neurology

The Future is Collaborative

The era of precision neurology is here, driven by a crucial partnership between neurologists and geneticists. Combining rich clinical phenotypes with rigorous molecular analysis is key.

Advanced Technologies

Leveraging LRS, RNA-Seq, and AI for for deeper insights.

Population-Specific Data

Grounding interpretations in local genetic context.

Interdisciplinary Partnership

Solving challenging cases through combined expertise.

By embracing advanced technologies and population-specific data, we can solve the most challenging cases and deliver on the promise of personalized promise of personalized medicine.

NGS in Pediatric Neurology

Exploring the transformative impact of Next-Generation Sequencing in diagnosing and treating pediatric neurological disorders.

Thank You