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Re-analysis of Exome Sequencing Data of Undiagnosed Epilepsy cases

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1. Introduction

Many types of epilepsies have a genetic aetiology. The number of single genes causing epilepsy is in the hundreds, and it is a number that continues to grow. A genetic diagnosis can have a major impact on a patient's clinical management in terms of treatment choice, avoiding unnecessary further testing and informing future reproductive decisions. The rapidly changing landscape of epilepsy genetics makes it essential to establish new strategies for genetic testing that aim to increase the diagnostic yield.

Salinas *et al.* (2021) demonstrated the importance of periodic re-analysis and re-interpretation of genetic data to achieve this. They showed that re-analysis of exome data of previously unsolved developmental and epileptic encephalopathy cases (complex paediatric epilepsies) increased the diagnostic yield by ~15%.

A recognised obstacle to periodic re-analysis of genetic data is the significant workload involved. The use of AI (Artificial Intelligence) is increasingly being used in genetic analysis and has been shown to successfully aid in well-timed re-analysis and help solve undiagnosed cases (Kadlubowska and Schrauwen 2022).

2. Objectives

1. To evaluate the diagnostic potential of exome sequence re-analysis in undiagnosed epilepsy cases.
2. To assess the accuracy and impact of Congenica-AI (Artificial Intelligence) for the analysis of SNVs.

3. Methods

Patient Selection

Re-analysis of exome sequencing was performed on a cohort of postnatal cases referred over an approximately 3-year period with a presentation of epilepsy (including the following terms on the referral card: epilepsy, seizures, infantile spasms, hypsarrhythmia and focal cortical dysplasia) as part of a service provided by Congenica and the South West Thames Regional Genetics Service, London, UK. The cohort included 93 probands and was comprised of 67 singletons, 3 duos and 23 trios.

Sequence Capture and Analysis

Exome sequencing was performed by hybridization using Agilent SureSelect Clinical Research Exome V2 (CRE V2) or Congenica ExomeCG and sequenced on Illumina NextSeq 500 or NovaSeq. Secondary and tertiary analysis of DNA sequences and review of SNVs and CNVs was undertaken using the Congenica clinical decision platform. Re-analysis was performed 6 - 48 months after initial interpretation, using 1) an updated curated epilepsy gene panel, and 2) gene agnostic prioritisation using Congenica AI.

4. Results

Through re-analysis a positive diagnosis was achieved In 17 out of 93 patients (18.3%).
Of 89 cases assessed for copy number variants (CNVs), 2 had pathogenic variants (2%).

CSNK2B	*DNM1 (2)*	*GABRA1*	***GRIA2***
HIVEP2	***IRF2BPL***	***KCNH1***	*KCNQ3*
NBEA	***NRROS***	***PCDH19***	*SCN1A*
SLC6A1	*SPAST*	*STXBP1*	***UGDH***

Table 1: The 16 diagnostic genes identified in the positive cases. Newly identified variants in genes are highlighted in bold, variants originally classified as VUS and reclassified as likely pathogenic/pathogenic are underlined. (n) = number of diagnoses, if greater than 1.

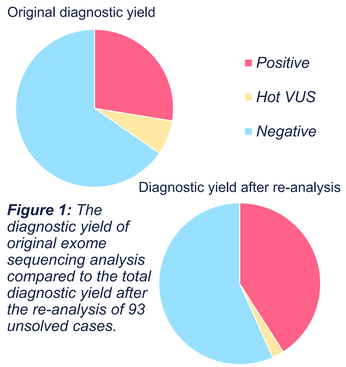


Figure 1: *The diagnostic yield of original exome sequencing analysis compared to the total diagnostic yield after the re-analysis of 93 unsolved cases.*

Re-analysis of previously unsolved epilepsy cases yielded a positive diagnosis in 17/93 cases (18.3%). This increases the overall diagnostic yield of the original cohort from 27.6% to 40.9%.

Of the 16 causal variants identified in 17 positive cases, 8 were newly identified and 9 had originally been reported as VUSs. 15/17 of the positive cases were singleton analysis. Most newly identified variants (5/8) are in genes that have only recently been reported to be associated with epilepsy. The remaining 3 cases had not previously had an epilepsy panel analysed (targeted singleton analysis) or had a CNV (CNV testing was only recently introduced).

The re-classification of VUSs was based on new evidence published after the original analysis. Referring clinicians were contacted after the re-analysis and confirmed that a diagnosis has been made in 5/9 of the VUS cases based on parental studies or functional work.

Congenica-AI correctly identified 12/14 (85.7%) causative SNVs (within the top 10 variants) in this undiagnosed cohort.

5. Discussion

This study illustrates the importance of re-analysis of cases without a genetic diagnosis, specifically in undiagnosed epilepsy cases. Through original exome sequencing analysis, a diagnosis was achieved in 35/129 cases (27.6%). After re-analysis, an additional 17/93 (18.3%) cases were found to have a causative variant and were re-categorised as diagnosed. This increases the overall diagnostic yield to 40.9%. Our results show a performance similar to that reported by Salinas *et al.* for developmental and epileptic encephalopathy cases.

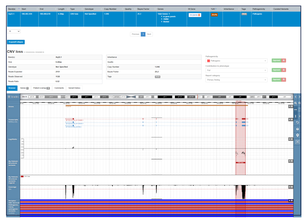
Congenica-AI accurately identified 85.7% of causative SNVs (within the top 10 variants) in this undiagnosed cohort. Read about the impact Congenica-AI had in the diagnosis of a *KCNH1* epilepsy case: https://genomics.congenica.com/ai-clinical-case-study.
The successful application of Congenica-AI in this re-analysis cohort demonstrates the value of this tool for periodic re-analysis of undiagnosed cases. Using Congenica-AI provides an efficient approach that makes routine re-analysis much more feasible.

Case Studies – Providing a Diagnosis

Case 1: *UGDH compound heterozygote*

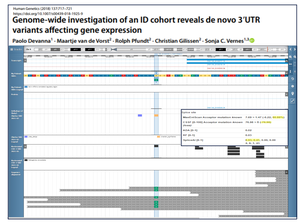
- Male singleton with infantile spasms and severe delay
- Compound heterozygous pathogenic variants identified in the *UGDH* gene: p.(Arg65Ter) + p.(Ala82Thr)
- Biallelic mutations in *UGDH* were first reported to cause developmental epileptic encephalopathy in 2020 (this patient was originally tested in 2018)
- Result consistent with a diagnosis of DEE84
- Recurrence risk is 1 in 4

Case 2: *PCDH19* deletion



- Female singleton with multifocal seizure disorder (GTCS, focal, absence) and global developmental delay.
- Deletion of *PCDH19* exons 1-3 identified.
- CNV analysis was introduced in 2021 (patient was originally tested in 2019)
- Consistent with a diagnosis of DEE9
- PCDH19 epilepsy is often pharmacoresistant. A diagnosis may help tailor treatment to use the most effective drugs (Dell'Isola *et al.* 2022)

Case 3 – Recurrent *DNM1* splice variant



- A *DNM1* splice site variant was identified twice in our cohort: c.1335+1638G>A (c.1197-8G>A in alternative transcript).
- Originally identified in 2019 as a VUS.
- Splice studies have shown that it has an impact on splicing (personal communication with clinician).
- Subsequently published as a pathogenic recurrent splice site variant (Sahly *et al.* 2020; Ruggiero *et al.* 2021).
- This variant has been re-classified and a diagnosis confirmed in both patients.

References: Salinas *et al.* (2021) Clinical next generation sequencing in developmental and epileptic encephalopathies: Diagnostic relevance of data re-analysis and variants re-interpretation'. Eur. J. Med. Genet. 64(12):104363
Dell'Isola *et al.* (2022) The Broad Clinical Spectrum of Epilepsies Associated With Protocadherin 19 Gene Mutation. Front Neurol. 12:780053. doi:10.3389/fneur.2021.780053
Sahly *et al.* (2020) Severe DNM1 encephalopathy with dysmyelination due to recurrent splice site pathogenic variant. Hum Genet. 139(12) 1575-1578
Ruggiero *et al* (2021) DNM1-Related Disorders: A Recurrent Splice Variant in an Alternative Transcript Expands the Genetic and Phenotypic Spectrum [Conference presentation abstract]. American Epilepsy Society. Submission ID: 1826335
Kadlubowska and Schrauwen (2022) Methods to Improve Molecular Diagnosis in Genomic Cold Cases in Pediatric Neurology. Genes (Basel). 13(2):333.

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