RESEARCH ARTICLE OPEN ACCESS

Holistic Exome-Based Genetic Testing in Adults With Epilepsy

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Abstract

Background and Objectives

Exome sequencing (ES) is increasingly used in the diagnostic workup of epilepsies. While its utility has been extensively demonstrated in children, its role in adults remains to be defined. In this study, we evaluate the outcomes of a holistic exome-based approach in adults with epilepsy.

Methods

We included 106 adults with epilepsy and a presumed genetic etiology between January 2015 and December 2023 at the Medical University of Vienna, Austria. Diagnostic ES, including copy number variation (CNV) and mitochondrial analyses, was performed. We report on diagnostic outcomes, phenotype expansions, and research findings. Furthermore, we compared the diagnostic outcomes with 3 comprehensive gene panels.

Results

In our cohort, the diagnostic yield was 30.2%, outperforming all 3 simulated gene panels. A developmental and epileptic encephalopathy phenotype was associated with receiving a genetic diagnosis. Overall, 27 distinct molecular etiologies were identified. Eight patients had pathogenic CNVs, and 2 had mitochondrial DNA variants. Molecular diagnoses had potential clinical implications in 8 of 32 solved cases (25%), which were eventually exerted in 5 patients (15.6%). Tailored treatment changes were successfully applied in *SCN1A*-related epilepsy (discontinuation of sodium channel blockers) and GLUT1 deficiency (ketogenic diet). Three patients with mitochondrial diseases were referred for preventive screening investigations after the genetic diagnosis. Our findings expand the clinical spectrum of 3 known epilepsy genes. In addition, explorative variant prioritization identified heterozygous truncating variants in *CLASP1* in 2 unrelated patients with focal epilepsy, suggesting it as a candidate gene.

Discussion

Our study strongly supports the use of holistic genetic approaches, encompassing CNV and mitochondrial analyses, in adults with epilepsy. Similar to pediatric cohorts, results may inform clinical care. Moreover, we report on phenotype expansions and a candidate gene discovery.

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Introduction

Epilepsy is a debilitating neurologic disorder with a lifetime prevalence of around 1%–2%, characterized by a chronic predisposition to unprovoked seizures.[1] Genome-wide case-control studies have revealed a substantial hereditary contribution to epilepsy, underscoring the role of

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Glossary

ACMG = American College of Medical Genetics and Genomic; **ASM** = anti-seizure medication; **CNV** = copy number variation; **DEE** = developmental and epileptic encephalopathy; **ES** = exome sequencing; **FS** = febrile seizure; **GGE** = genetic generalized epilepsy; **GTCS** = generalized tonic-clonic seizures; **ID** = intellectual disability; **LFE** = lesional focal epilepsy; **MAF** = minor allele frequency; **mtDNA** = mitochondrial DNA; **NAFE** = nonacquired focal epilepsy; **NGS** = next-generation sequencing; **SNV** = single nucleotide variant.

common and rare genetic variants.[2,3] Moreover, large-scale genetic investigations have highlighted the involvement of different variant types, such as rare single nucleotide variants (SNVs) and copy number variants (CNVs), in various classes of epilepsy.[4-6] While most cases exhibit a polygenic background, a smaller but significant portion is caused by monogenic defects, making them amenable to genetic testing.[7]

Over the past decade, next-generation sequencing (NGS) has entered clinical practice and is now used in the routine diagnostic workup for patients with epilepsy of suspected genetic origin.[8] A meta-analysis revealed an overall diagnostic yield exceeding 20%, with the rate varying markedly depending on the type of testing and patient selection.[9] Yet, the use of NGS has predominantly been evaluated in pediatric cohorts,[10,11] while less is known about its utility in adults. The first studies on adults with epilepsy and intellectual disability (ID) found that the outcomes may be comparable with those in pediatric populations.[12-16] This is supported by a study on adults with developmental and epileptic encephalopathies (DEEs), yielding genetic diagnoses in more than 25% of cases.[17] Notably, monogenic causes can also be identified in 12% of patients with nonacquired focal epilepsy (NAFE).[18,19] By contrast, a trio exome-based study on genetic generalized epilepsy (GGE) yielded a rate of less than 2%, most likely because of a predominance of polygenic contributions.[20]

In a clinical setting, molecular findings from NGS can have direct management implications. A genetic diagnosis may influence the use or avoidance of specific treatments, including antiseizure medication (ASM) and a ketogenic diet.[21] Anecdotal evidence also suggests that genetic testing can inform the selection of epilepsy surgery candidates.[22]

A limitation of many sequencing studies is their focus on SNVs. However, a substantial number of monogenic epilepsy syndromes are associated with CNVs or mitochondrial variants.[4,23,24] These genetic alterations may be overlooked by targeted gene panels, thus limiting diagnostic outcomes. Consistent with this, it has been demonstrated that unbiased approaches achieve higher yields than commercial gene panels.[16]

In this study, we report our experiences with a holistic exome-based approach, including CNV and mitochondrial analyses, in a comprehensively phenotyped cohort of 106 adults with different epilepsies. In addition, we report on the clinical utility, phenotype expansions, and a new candidate gene discovery.

Methods

Patient Selection

All patients aged 18 years and older with unexplained epilepsy, who were evaluated at the Department of Neurology of the Medical University of Vienna, Austria, and underwent diagnostic exome sequencing (ES) between January 2015 and December 2023 were included in this retrospective study. All patients received standard clinical, electrophysiologic and imaging evaluation at the discretion of the treating physician. Genetic testing was performed as part of the clinical workup with the indication provided by a consultant epileptologist, based on international guidelines.[25] Clinical details as well as findings from (video) EEG and neuroimaging were derived from the patients' in-house records (i.e., retrospective chart review). Based on these findings, patients were grouped into 5 diagnostic categories: (1) GGE, (2) NAFE, (3) lesional focal epilepsy (LFE), (4) DEE, and (5) unclassified epilepsy.

Exome Sequencing and Data Analysis

ES was performed at the Institute of Human Genetics (Technical University of Munich, Germany) using a SureSelect Human All Exon Kit (Agilent, 50 mb V5 or Agilent 60 mb V6) or a Twist Human Exome 2.0 Plus Comprehensive Exome Spike-in and Mitochondrial Panel for enrichment. Sequencing was performed on an Illumina HiSeq2500, HiSeq4000, or NovaSeq6000 system (Illumina, San Diego, CA).

Two bioinformatic pipelines were used: (1) SNVs and small insertions/deletions were initially analyzed using SAMtools.[26] (2) The second analysis was conducted using the Genome Analysis Toolkit (GATK) HaplotypeCaller pipeline.[27] ExomeDepth and Pindel were applied for the detection of CNVs.[28,29] Mitochondrial DNA (mtDNA) was analyzed using off-target reads, as previously described.[30] Variants were filtered based on minor allele frequency (MAF), which was estimated using our in-house database and confirmed by Genome Aggregation Database (gnomAD), i.e. < 0.1% for recessive and < 0.01% for dominant filters. Variants were classified according to the standards of the American College of Medical Genetics and Genomics (ACMG).[31] Reported missense variants were retrospectively evaluated using AlphaMissense.[32] Actionable findings (unrelated to epilepsy) were reported in line with the ACMG guidelines for secondary findings.[33]

To prioritize candidate genes, we searched for rare (MAF < 0.01%) nonsynonymous variants in each exome data set. Variants were

manually screened using in silico prediction, gnomAD constraint metrics, and a literature review focusing on previously published cases. Such prioritization is used to identify promising candidate variants without being comprehensive.

In our clinical routine, exome data sets and variants are not systematically re-evaluated on a regular basis. However, cases may be reanalyzed or reassessed individually based on clinical needs and the results of genetic assessments. This process may also involve multidisciplinary case discussions, typically including epileptologists and geneticists. For this study, all reported VUS were cross-referenced with the ClinVar database, and their classifications were updated accordingly.

Statistical Analysis

The rate of pathogenic and likely pathogenic variants in the cohort (compatible with the phenotype and mode of inheritance) established the diagnostic yield. Demographic and clinical baseline characteristics were analyzed descriptively.

Furthermore, we sought to identify clinical and demographic factors associated with a molecular diagnosis. For univariate comparison, Fisher exact test (2-sided) was used for categorical data and Mann-Whitney *U* test for continuous data. Owing to the explorative nature of the study, correction for multiple testing was not applied.

Multivariable logistic regression was used to investigate which factors are associated with receiving a genetic diagnosis. Covariates included age at epilepsy onset, epilepsy syndrome, family history, comorbid ID/developmental delay (DD), history of febrile seizures (FS), ASM resistance, and MRI findings. A p value < 0.05 was considered statistically significant. SPSS v29.0 (IBM, Chicago) was used for statistical analyses. Figures were created using GraphPad Prism v10.

Comparison with Simulated Gene Panels

We investigated, whether our obtained single-gene etiologies would have been covered by virtual epilepsy gene panels of different extent to simulate their diagnostic performance. Known epilepsy-related microdeletion or microduplication syndromes that do not involve monogenic disease genes were not included in this analysis. Panel contents were taken from the providers' websites on July 18, 2024. We selected the GeneDx Comprehensive Epilepsy Gene Panel (144 genes), Blueprint Comprehensive Epilepsy Panel (511 genes), and Fulgent Epilepsy Comprehensive NGS panel (1,057 genes). The detailed panel contents are provided as supplementary material (eAppendix 1).

Standard Protocol Approvals, Registrations, and Patient Consents

The study was performed in concordance with the principles of the Declaration of Helsinki and approved by the ethics committee of the Medical University of Vienna (EC-Nr. 1021/2018). Informed consent was obtained from all included patients or their legal guardians before inclusion.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Statement on the Use of Artificial Intelligence

The authors used the ChatGPT (version GPT-4) language model provided by OpenAI for language optimization in this study.

Results

Demographic and Clinical Characteristics

We analyzed 106 patients who underwent diagnostic ES. Forty-nine patients (46.2%) were male, and 57 (53.8%) were female. The median age at seizure onset was 12 years (IQR 3–17). A total of 21.7% of the patients experienced seizure onset in adulthood, whereas almost one half (48.1%) had their first seizure during their first decade of life. Brain MRI results were unremarkable in 44.3%, showed unspecific abnormalities in 30.2%, and revealed epilepsy-related lesions in 25.5%. The most common epilepsy diagnosis in our cohort was NAFE (30.2%), followed by GGE (21.7%), DEE (17%), LFE (16%), and unclassified epilepsy (15.1%). A family history of seizures or epilepsy was reported in more than one-third (34.9%) of the patients. In addition, 17% had a history of FS and more than one half (52.8%) had comorbid ID/DD. Most of the patients (71.7%) were refractory to ASM treatment, with 10.4% undergoing epilepsy surgery and 13.2% receiving vagus nerve stimulation. Clinical and demographic cohort characteristics are summarized in Table 1.

Genetic Findings

Ninety-eight of the 106 patients (92.5%) were sequenced as singletons and 8 as trios (7.5%). The diagnostic rate in our study was 30.2%, with 33 genetic diagnoses established in 32 cases (i.e., 1 patient with a dual diagnosis). Overall, 27 distinct molecular etiologies were identified. Of the 33 (likely) pathogenic variants, 15 were missense variants (45.5%), 8 CNVs (24.2%), 6 nonsense (18.2%), and 4 frameshift (12.1%). In solved cases, the mode of inheritance was autosomal dominant in 26 (78.8%), X-linked in 3 (9.1%), and autosomal recessive and mitochondrial in 2 cases (6.1%) each. The spectrum of underlying gene etiologies and variant types is illustrated in the Figure. The core clinical, demographic, and molecular features of genetically resolved cases are listed in Table 2. Case vignettes including variant details are provided as supplementary material (eAppendix 2).

Five of the above solved cases were initially reported as nondiagnostic (i.e., either VUS or no relevant variant) by the genetic laboratory but were subsequently solved through genotype-guided reassessment or exome data reanalysis. This process involved upgrading variants from VUS to (likely)

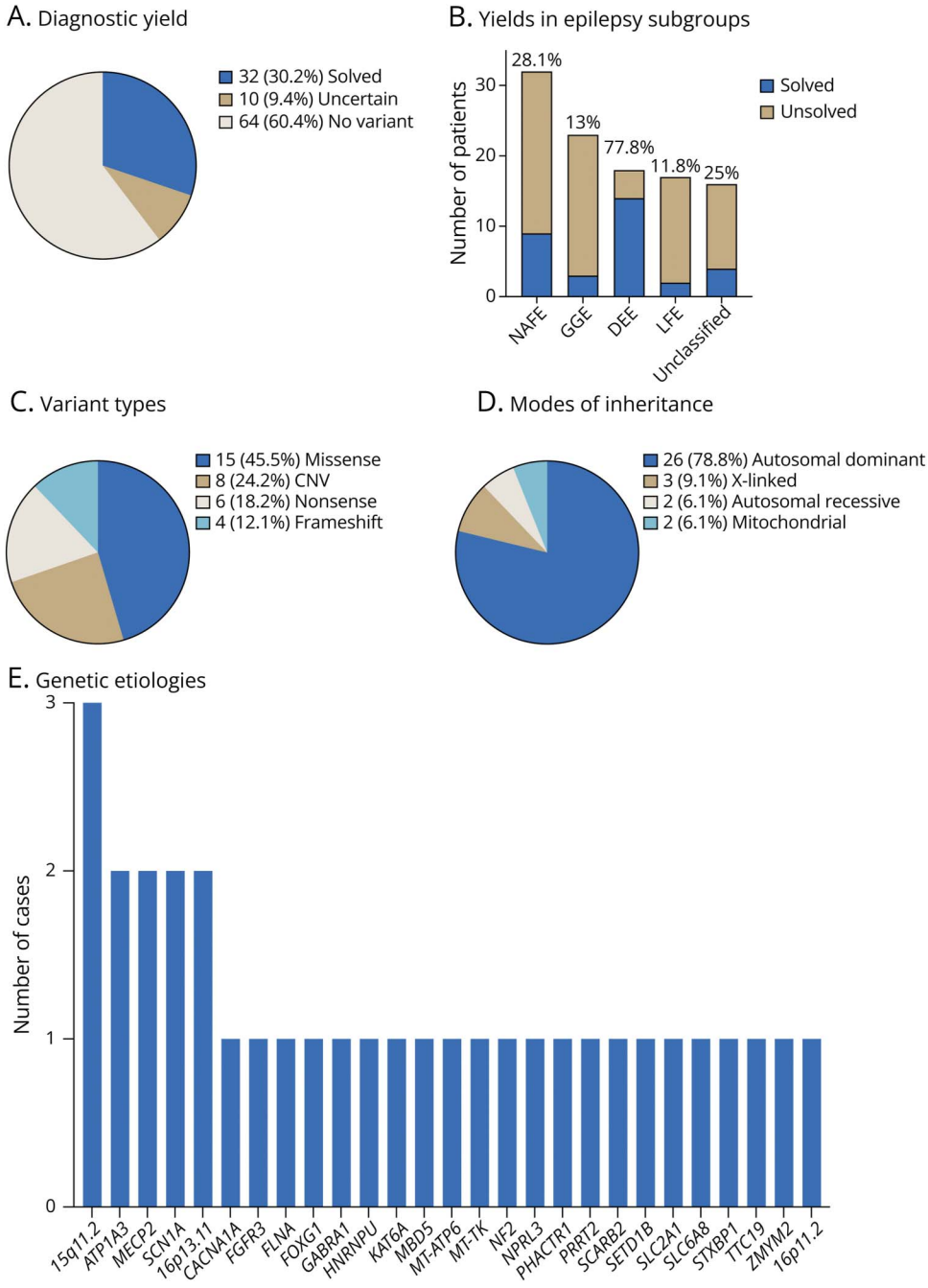
Table 1 Demographic and Clinical Features of Genetically Solved Compared With Unsolved Cases

Characteristics	Total cohort n = 106, (%)	Genetically solved n = 32, (%)	Genetically unsolved n = 74, (%)	*p* Value
Sex				0.833
Male	49 (46.2)	14 (43.8)	35 (47.3)	
Female	57 (53.8)	18 (56.3)	39 (52.7)	
Median age at seizure onset (range)	12 y (0–42 y)	8 y (0–42 y)	12 y (0–42 y)	0.398
Type of epilepsy				<0.001[a]
NAFE	32 (30.2)	9 (28.1)	23 (31.1)	
GGE	23 (21.7)	3 (9.4)	20 (27.0)	
DEE	18 (17.0)	14 (43.8)	4 (5.4)	
LFE	17 (16.0)	2 (6.3)	15 (20.3)	
Unclassified	16 (15.1)	4 (12.5)	12 (16.2)	
Brain MRI				0.332
Normal	47 (44.3)	16 (50.0)	31 (41.9)	
Unspecific	32 (30.2)	11 (34.4)	21 (28.4)	
Epilepsy-related lesion(s)	27 (25.5)	5 (15.6)	22 (29.7)	
Family history				1
Positive	37 (34.9)	11 (34.4)	26 (35.1)	
Negative	69 (65.1)	21 (65.6)	48 (64.9)	
History of FS				0.782
Yes	18 (17.0)	6 (18.8)	12 (16.2)	
No	88 (83.0)	26 (81.3)	62 (83.8)	
Concomitant ID/DD				0.094
Yes	56 (52.8)	21 (65.6)	35 (47.3)	
No	50 (47.2)	11 (34.4)	39 (52.7)	
ASM resistance				0.625
Yes	76 (71.7)	21 (65.6)	55 (74.3)	
No	17 (16.0)	6 (18.8)	11 (14.9)	
Undefined	13 (12.3)	5 (15.6)	8 (10.8)	
Epilepsy surgery				1
Yes	11 (10.4)	3 (9.4)	8 (10.8)	
No	95 (89.6)	29 (90.6)	66 (89.2)	
VNS				0.756
Yes	14 (13.2)	5 (15.6)	9 (12.2)	
No	92 (86.8)	27 (84.4)	65 (87.8)	

Abbreviations: ASM = antiseizure medication; DD = developmental delay; DEE = developmental and epileptic encephalopathy; FS = febrile seizure; GGE = genetic generalized epilepsy; ID = intellectual disability; LFE = lesional focal epilepsy; NAFE = nonacquired focal epilepsy; VNS = vagus nerve stimulation.
[a] Statistically significant.

pathogenic in *SLC2A1* (assessment of CSF glucose), *FGFR3* (additional information available), *GABRA1*, and *PHACTR1* (secondary de novo confirmation). In addition, exome data reanalysis successfully resolved 1 case (*MBD5*) where no variant had initially been reported. In addition to (likely) pathogenic variants, 11 variants of uncertain significance

Figure Genetic Findings in Our Cohort of 106 Adults With Epilepsies



(A) Overall diagnostic yield of the investigated cohort. (B) Different diagnostic rates in different phenotypic subgroups. (C) Types of genetic variations in diagnostic cases. (D) Observed modes of inheritance in solved cases. (E) Underlying genetic etiologies in patients with a molecular diagnosis. CNV = copy number variation; DEEs = developmental and epileptic encephalopathies; GGE = genetic generalized epilepsy; LFE = lesional focal epilepsy.

(VUS) in 9 different genes potentially relevant to the phenotype were reported in 10 cases, i.e., 9.4% (genes and variants listed in Table 3).

A total of 93.3% of epilepsy-related missense variants that were classified as (likely) pathogenic according to ACMG standards were correctly predicted to be pathogenic by AlphaMissense, while only 1 was predicted as ambiguous and none as benign. By contrast, only 22.2% of the missense VUS were predicted as pathogenic, whereas 33.3% were predicted as ambiguous and 44.4% as benign.

Actionable variants in genes unrelated to the epilepsy phenotype (i.e., secondary findings) were reported in 6 cases (5.7% of the cohort), including the genes *BRCA1*, *BRCA2*, *HNF1A*, *KCNH2*, *KCNQ1*, and *PALB2*.

Factors Associated With a Molecular Diagnosis

Our univariate analysis revealed a statistically significant difference between genetically solved and unsolved cases concerning epilepsy syndrome ($p < 0.001$), while all other investigated parameters were non-significant (Table 1). A subsequently performed multivariable logistic regression

Table 2 Demographic, Clinical, and Molecular Details of the 33 Cases With a Molecular Diagnosis

Patient number	Age group at seizure onset[a]	Epilepsy diagnosis	Seizure types	EEG	MRI	Intellectual disability	Gene (Transcript) Variant(s)	AlphaMissense prediction (score)	Variant classification	Case published previously (PMID)
3[b]	Infantile	DEE	Atypical absences, atonic, myoclonic	GSW, GPSW	Microcephaly, corpus callosum dysgenesis	Yes	*SLC2A1* NM_006516.4: c.1234T>G, p.(Trp412Gly) heterozygous	Pathogenic (0.972)	LP	N/A
7	Infantile	DEE	Unknown	Not performed	Normal	Yes	NM_152296.5 c.2401G>A, p.(Asp801Asn) Heterozygous	Pathogenic (0.987)	P	32339621
9	Infantile	NAFE	Focal impaired awareness, atonic	Interictal: Multifocal spikes, ictal: Right temporal/central rhythmic activity	Hypoplasia right superior frontal gyrus	Yes	15q11.2 microdeletion (chr15: 22833525-23313293) heterozygous	N/A	P	N/A
11	Adult	GGE	Myoclonic, GTCS	Diffuse background slowing	Normal	No	NM_005506.4 c.134del, p.(Asn45Metfs*88) Homozygous	N/A	P	31407473
16	Infantile	DEE	Tonic	GPFA	Normal	Yes	*STXBP1* NM_001032221.6: c.308A>C, p.(His103Pro) heterozygous	Pathogenic (0.996)	LP	N/A
17[b]	Childhood	DEE	Atonic, absence, GTCS	GSW, GPSW	Normal	Yes	*GABRA1* NM_001127644.2: c.541C>T, p.(Pro181Ser) heterozygous (*de novo*)	Pathogenic (0.776)	LP	31568673
19	Childhood	DEE	Unknown	Interictal: Normal, ictal: Not performed	Normal (CT)	Yes	*ATP1A3* NM_152296.5: c.2332A>C, p.(Thr778Pro) heterozygous	Pathogenic (0.966)	LP	32339621
23	Infantile	DEE	Absence, GTCS	GSW	Normal	Yes	*SETD1B* NM_001353345.2: c.5828A>G, p.(Tyr1943Cys) heterozygous (*de novo*)	Pathogenic (0.998)	P	35385430
24	Adult	Unclassified	GTCS	Diffuse slowing, NCSE	Periventricular and basal ganglia hyperintensities, cerebellar atrophy	Yes	*TTC19* NM_017775.4: c.554T>C, p.(Leu185Pro) homozygous	Pathogenic (0.994)	LP	N/A
29	Infantile	DEE	GTCS, tonic/atonic	Diffuse slowing, multifocal spikes	Left temporal FCD	Yes	*HNRNPU* NM_031844.3: c.575C>A, p.(Ser192*) heterozygous	N/A	P	N/A
30	Infantile	NAFE	Focal aware, focal impaired awareness, BTCS	Interictal: Diffuse slowing, multifocal spikes, ictal: Right hemispheric rhythmic activity	Normal	Yes	*SCN1A* deletion (exons 6-7) (chr2:166905416-166908632) heterozygous	N/A	P	N/A
31	Childhood	LFE	Focal aware (motor), focal impaired awareness, BTCS	Interictal: Left frontal spikes, ictal: Rhythmic activity left frontocentral	Left frontal lesion (histology: Meningioma, intracortical microhamartomas)	Yes	*NF2* NM_000268.4: c.784C>T, p.(Arg262*) heterozygous (mosaic)	N/A	P	N/A

Continued

Table 2 Demographic, Clinical, and Molecular Details of the 33 Cases With a Molecular Diagnosis *(continued)*

Patient number	Age group at seizure onset[a]	Epilepsy diagnosis	Seizure types	EEG	MRI	Intellectual disability	Gene (Transcript) Variant(s)	AlphaMissense prediction (score)	Variant classification	Case published previously (PMID)
32	Adolescence	Unclassified	Myoclonic, impaired awareness, GTCS	GSW, left frontal spikes	Normal	No	16p13.11 microdeletion (chr16: 15045472-16359035) heterozygous	N/A	P	N/A
33	Childhood	LFE	Focal aware, focal impaired awareness, BTCS	Interictal: Intermittent slowing, ictal: Left hemispheric rhythmic activity	Bilateral PVNH	No	*FLNA* NM_001110556.2: c.5363_5369del, p.(Leu1788Profs*39) heterozygous	N/A	P	N/A
34	Childhood	DEE	Tonic, atypical absences, GTCS	Interictal: Diffuse slowing, GPS, GSW, slow-spike-waves, GPFA, ictal: Bilateral rhythmic delta	Microcephaly, corpus callosum atrophy, leukoencephalopathy, vermian/motor cortex atrophy	Yes	*MECP2* duplication (chrX: 152935912–153609556) hemizygous	N/A	P	N/A
36[b]	Adult	NAFE	Focal impaired awareness, BTCS	Interictal: Bitemporal spikes, ictal: Rhythmic activity (different lateralization)	Normal	Yes	*MBD5* NM_001378120.1: c.1690C>T, p.(Gln564*) heterozygous	N/A	LP	N/A
43	Childhood	Unclassified	Focal impaired awareness, BTCS	Intermittent slowing	Cerebellar atrophy	No	*CACNA1A* NM_001127222.2: c.1994C>T, p.(Thr665Met) heterozygous	Pathogenic (0.972)	P	N/A
49[b]	Adult	NAFE	Focal aware, focal impaired awareness, BTCS	Interictal: Bitemporal spikes, ictal: Right/left temporal rhythmic activity	HS right	No	*FGFR3* NM_000142.5: c.1612A>G, p.(Ile538Val) heterozygous	Ambiguous (0.465)	P	N/A
50	Infantile	NAFE	Focal impaired awareness, BTCS	Normal	Normal	No	*NPRL3* NM_001077350.3: c.898C>T, p.(Gln300*) heterozygous	N/A	P	32086284
52	Childhood	NAFE	Focal impaired awareness, BTCS	Right temporal spikes	Mild atrophy, leukoencephalopathy	Yes	*SLC6A8* NM_005629.4: c.1145C>T, p.(Pro382Leu) heterozygous	Pathogenic (0.966)	P	N/A
54	Childhood	DEE	Atypical absence	Generalized slowing, GSW, multifocal spikes	Diffuse atrophy	Yes	*KAT6A* NM_006766.5: c.4228_4232del, p.(Lys1410Glyfs*7) heterozygous (*de novo*)	N/A	P	N/A
55	Childhood	DEE	GTCS	Diffuse slowing	Normal	Yes	*SCN1A* NM_001165963.4: c.4279C>T, p.(Gln1427*) Heterozygous	N/A	P	N/A
							ZMYM2 NM_003453.6: c.1309G>T, p.(Glu437*) heterozygous	N/A	LP	N/A

Continued

Table 2 Demographic, Clinical, and Molecular Details of the 33 Cases With a Molecular Diagnosis *(continued)*

Patient number	Age group at seizure onset[a]	Epilepsy diagnosis	Seizure types	EEG	MRI	Intellectual disability	Gene (Transcript) Variant(s)	AlphaMissense prediction (score)	Variant classification	Case published previously (PMID)
68	Adult	NAFE	Focal aware, focal impaired awareness, BTCS	Interictal: Right temporal spikes, ictal: Right temporal rhythmic activity	Meningioma (not related to seizures)	Yes	15q11.2 microduplication (chr15: 22835916-23086410) heterozygous	N/A	P	N/A
69[b]	Infantile	DEE	Tonic, myoclonic, absences, GTCS	Interictal: Generalized slowing, GSW, multifocal spikes, ictal: Rhythmic activity bilateral/GSW	Subcortical gliosis (unspecific)	Yes	*PHACTR1* NM_030948.6: c.209C>G, p.(Pro70Arg) heterozygous (*de novo*)	N/A	LP	N/A
75	Adult	Unclassified	GTCS	Normal	Normal	No	*PRRT2* NM_145239.3: c.457_458del, p.(Lys153Alafs*16) heterozygous	N/A	LP	N/A
78	Adult	NAFE	GTCS	Generalized and multifocal spikes	Normal	No	16p11.2 microduplication (chr16: 29675050-30206111) heterozygous	N/A	P	N/A
82	Adult	GGE	Myoclonic, absences, GTCS	GSW	Normal	No	*MT-TK* NC_012920.1: m.8344A>G heteroplasmy 80%	N/A	P	N/A
86	Adolescence	NAFE	Focal aware, focal impaired awareness, BTCS	Interictal: Right frontal spikes, ictal: Rhythmic activity right frontal	Normal	No	16p13.11 microdeletion (15045882_15069057) _(16359035_17202553) heterozygous	N/A	P	N/A
88	Childhood	DEE	Focal impaired awareness, GTCS	Multifocal spikes, GPFA	Microcephaly	Yes	*FOXG1* NM_005249.5: c.800G>A, p.(Gly267Asp) heterozygous	Pathogenic (1)	P	N/A
93	Adolescence	GGE	Myoclonic, GTCS	GSW, GPS	Basal ganglia signal alterations, cerebellar atrophy	No	*MT-ATP6* NC_012920.1: m.8993T>C heteroplasmy 95%	N/A	P	N/A
95	Childhood	DEE	Atypical absences, GTCS	Generalized slowing, generalized spikes	Normal	Yes	*MECP2* NM_001110792.2: c.961C>T, p.(Arg321Trp) heterozygous	Pathogenic (0.981)	P	N/A
99	Infantile	DEE	Tonic, atonic, atypical absences, GTCS	GSW, GPFA	Mild atrophy	Yes	15q11.2 microduplication (chr15: 23684690-28600151) heterozygous	N/A	P	N/A

Abbreviations: BTCS = bilateral tonic-clonic seizure; DEE = developmental and epileptic encephalopathy; GGE = genetic generalized epilepsy; LFE = lesional focal epilepsy; EEG = electroencephalography; FCD = focal cortical dysplasia; GGE = genetic generalized epilepsy; GPFA = generalized paroxysmal fast activity; GPS = generalized polyspikes; GPSW = generalized polyspike and waves; GSW = generalized spike and waves; GTCS = generalized tonic-clonic seizures; HS = hippocampal sclerosis; LFE = lesional focal epilepsy; LP = likely pathogenic; N/A = not applicable; NAFE = nonacquired focal epilepsy; NCSE = nonconvulsive status epilepticus; P = pathogenic; PMID = PubMed ID; PVNH = periventricular nodular heterotopia.

[a] Age ranges: infantile: 0–2 years, childhood: 3–12 years, adolescence: 13–17 years, adult: ≥18 years.

[b] Case resolved through reassessment/reanalysis.

Table 3 Rare and Potentially Epilepsy-Related Variants of Uncertain Significance (VUS) in the Cohort

Patient ID	Epilepsy syndrome	AlphaMissense prediction (score)	Gene (Transcript) Variant(s)	Allele frequency (gnomAD)	ClinVar classification[a]
2	LFE	*AFF2* NM_002025.4: c.43C>T, p.(Gln15*) Heterozygous	N/A	0	N/A
3	DEE	*SPTAN1* NM_001130438.3: c.4552G>A, p.(Ala1518Thr) heterozygous	Benign (0.328)	0	Likely benign
5	NAFE	*GRIN2A* NM_001134407.3: c.56G>A, p.(Arg19His) heterozygous	Benign (0.143)	0	N/A
6	GGE	*SCN9A* NM_001365536.1: c.4009A>C, p.(Ile1337Leu) heterozygous	Ambiguous (0.593)	<0.01%	Uncertain significance(1); likely benign(1)
		SCN9A NM_001365536.1: c.785T>C, p.(Ile262Thr) heterozygous	Ambiguous (0.341)	<0.01%	Uncertain significance
10	LFE	*GRIN2A* NM_001134407.3: c.3902A>G, p.(Glu1301Gly) heterozygous	Benign (0.124)	0	Uncertain significance
38	GGE	*PRRT2* NM_145239.3: c.818T>A, p.(Ile273Asn) heterozygous	Pathogenic (0.995)	0	N/A
51	Unclassified	*SPTBN1* NM_003128.3: c.1728C>A, p.(Asp576Glu) heterozygous	Pathogenic (0.934)	0	N/A
65	GGE	*PTRHD1* NM_001013663.2: c.365G>A, p.(Arg122Gln) homozygous	Ambiguous (0.371)	<0.01%	Uncertain significance
76	NAFE	*SETD1B* NM_001353345.2: c.17_25del, p.(Pro6_His8del) heterozygous	N/A	0	Uncertain significance
81	LFE	*SCN3A* NM_006922.4: c.3706A>G, p.(Ile1236Val)	Benign (0.07)	<0.01	Uncertain significance

Abbreviations: DEE = developmental and epileptic encephalopathy; GGE = genetic generalized epilepsy; LFE = lesional focal epilepsy; NAFE = nonacquired focal epilepsy.
[a] Accessed on December 6, 2024.

(Nagelkerke pseudo-R squared: 0.245) showed that an epilepsy phenotype classified as DEE increased the likelihood of receiving a molecular diagnosis (odds ratio 6.42; 95% CI 1.61–25.64).

Exome Sequencing vs Simulated Gene Panels

Overall, our diagnostic findings include 27 molecular etiologies, 24 of which are single-gene etiologies and 3 known microdeletion and duplication syndromes. The GeneDx Comprehensive Epilepsy Gene Panel (144 genes) covers 15 of 24 single gene etiologies, but do not include *FGFR3*, *KAT6A*, *MT-ATP6*, *MT-TK*, *NF2*, *PHACTR1*, *SETD1B*, *TTC19*, and *ZMYM2*. The Blueprint Comprehensive Epilepsy Panel (511 genes) covers 20 of 24 disease genes. This panel also covers mitochondrial variants, but still misses *FGFR3*, *KAT6A*, *NF2*, and *ZMYM2*. The Fulgent Epilepsy Comprehensive NGS panel (1,057 genes) achieved a simulated rate of 19 of 24 genes, but did not include mitochondrial genes *MT-ATP6*, *MT-TK*, *NF2*, *TTC19*, and *ZMYM2*. A combination of all 3 panels includes 22 of 24 genes, but still does not cover 2 genes (*NF2* and *ZMYM2*).

Clinical Utility of Genetic Diagnoses

Molecular diagnoses with potential clinical utility were present in 8 patients, i.e., 25% of solved cases. Resulting management implications were eventually implemented in 5 patients (15.2% of solved cases).

1. In a patient with *SCN1A*-related focal epilepsy (due to a heterozygous 2-exon deletion predicted to result in a loss of function), discontinuation of sodium channel blockers led to a significant reduction in seizures. Because of reported poor responses to epilepsy surgery in genetic channelopathies, presurgical workup was also halted in this case.[22]

2. In a patient with GLUT1 deficiency (*SLC2A1*), a ketogenic/modified Atkins diet was initiated and led to a stabilization regarding seizure frequency, cognitive functioning, and general well-being.
3. The genetic diagnosis also led to a preventive screening for comorbidities (e.g., ophthalmology, cardiology, and endocrinology) in the 3 cases with mitochondrial epilepsies (*TTC19*, *MT-TK*, and *MT-ATP6*).[34]
4. A patient with a heterozygous truncating *SCN1A* variant (predicted to result in a loss of function) had a DEE phenotype (Dravet syndrome) and has therefore never been considered an epilepsy surgery candidate. Moreover, he has remained seizure free prior to the molecular diagnosis; thus, there was no need to adjust the ASM regimen.
5. A male patient with a causative *PRRT2* variant had experienced paroxysmal kinesigenic dyskinesia in childhood and adult-onset unclassified epilepsy with only 2 generalized tonic-clonic seizures (GTCSs). Given the excellent response to levetiracetam prior to genetic diagnosis, the medication has remained unchanged, although a favorable response to sodium channel blockers has been reported for *PRRT2*-related syndromes.[35]
6. One patient with an *SLC6A8*-related disorder has not undergone follow-up since genetic diagnosis. While the condition may benefit from creatine, arginine, and glycine supplementation, its influence on her disease course remains unknown.[36]

Phenotype Expansions

Some molecular diagnoses involved known epilepsy genes with limited data on the phenotypic spectrum, particularly regarding epilepsy. Some relevant expansions of the phenotypic spectrum have already been published (Table 2).

In summary, 1 phenotype expansion was found in the patient with *MBD5*-related disease, who had adult-onset NAFE and a presumably average premorbid IQ, further delineating the mild end of the clinical spectrum.[37] We also corroborate anecdotal evidence that *FGFR3* is associated with bitemporal epilepsy without clinically overt signs of skeletal dysplasia.[38,39] Moreover, we provide longitudinal follow-up of an individual with *PHACTR1*-related DEE into adulthood, demonstrating that treatment-refractory seizures may continue and neuropsychiatric issues (such as aggression and anxiety) may even first manifest later in life.[40]

Candidate Gene Discovery

Through screening for rare truncating (i.e., frameshift, nonsense, and splice-site) variants in genes under evolutionary constraint (i.e., gnomAD pLI = 1), we identified 2 heterozygous loss-of-function variants in *CLASP1* in 2 unrelated individuals (NM_015282.3: c.2761_2762del, p.Leu921Cysfs*8 and c.1178+1G>A, p?). Both had childhood-onset, treatment-refractory (MRI-negative) NAFE and neurodevelopmental issues. Families/parents were not available for de novo confirmation or segregation analysis.

So far, *CLASP1* has not been associated with a monogenic disease. However, its high expression in brain tissue and its involvement in microtubule dynamics during axon/neurite outgrowth makes it a good candidate gene for CNS phenotypes.[41] It is worthy of note that 2 truncating variants in *CLASP1* were also found in the international Epi25 cohort, both of which occurred in NAFE cases, while no truncating variants were observed in controls.[2] Most recently, a biallelic missense variant in *CLASP1* was proposed as the underlying cause of lissencephaly, DD, and childhood-onset seizures in a consanguineous multiplex family.[42]

Discussion

In our study, we investigated the outcomes of a comprehensive exome-based testing approach in a well-characterized cohort of 106 adults with various epilepsies and comorbidities. In general, there is still a high need for real-life studies in this field, as NGS becomes increasingly used in a clinical context, and the vast majority of diagnostic studies have been limited to pediatric populations.

We identified the underlying molecular etiology in over 30% of cases. Previous studies have reported an extremely wide range of diagnostic yields. This variability can primarily be explained by differences in patient selection and the testing applications used. However, our diagnostic rate is within the range reported by 2 meta-analyses.[9,43]

The only predictor of a molecular diagnosis in our study was a DEE phenotype. In this phenotypic subgroup, we observed an overwhelmingly high diagnostic rate of more than 77%, exceeding previous data.[17] Notably, a diagnosis of DEE in adulthood is often uncertain because the syndromes present more specifically in childhood and later converge into a similar phenotypic end point. The yields in NAFE (28.1%) and GGE (13%) are also higher compared with earlier studies on these subgroups.[18,20] This could, in part, be attributed to our dynamic yield, with new diagnoses emerging through different types of reassessments. Supporting this trend, prior research suggests that a periodic reanalysis of NGS data enhances diagnostic rates in epilepsy.[44] In addition, we cannot exclude a selection bias at a tertiary care center, where more severe cases with a higher likelihood of a monogenic cause are usually encountered. Moreover, compared with studies with lower yields, we also included NAFE and GGE patients with neurodevelopmental comorbidities, which may further increase the odds of identifying a genetic cause.

The identification of 27 different genetic etiologies in our cohort highlights the considerable molecular heterogeneity of epilepsies. Most gene etiologies were only observed once across the entire cohort. A current review indicates that more than 1,500 genes are implicated in epilepsy.[45] The still-expanding genetic landscape, along with the recognized

pleiotropy, underscores the role of comprehensive testing approaches such as exome or genome sequencing.

As primarily evidenced in pediatric cohorts, a molecular diagnosis may inform prognosis and clinical management, offering precision medicine approaches for various etiologies.[46] In our work, we found that in at least one quarter of solved cases, the genetic diagnosis had potential implications for clinical care (i.e., *SCN1A*, *SLC2A1*, *PRRT2*, *SLC6A8*, and mitochondrial diseases). This corresponds with prior research that examined the benefits of genetic testing in adults with epilepsy.[13] Yet, unlike in pediatric studies, the implementation of precision care was less frequent in our adult cohort. This disparity may stem from challenges such as higher rates of loss to follow-up, disease stabilization following trial-and-error approaches during the preceding diagnostic odyssey, or the limited familiarity of adult neurologists with targeted treatments, possibly leading to more cautious approaches.

Despite remarkable improvements over the past decade, most of our 106 cases still remain unsolved. This may be due to inherent methodologic limitations, including the insufficient detectability of intronic variants and repeat expansions that also play a pathogenic role in some epilepsies.[47] An increasing number of centers have implemented whole-genome sequencing and long-read technologies to overcome this issue.[48,49] Second, some variants related to epileptogenic brain lesions are present in a mosaic state, while NGS from blood cells remains negative.[50] Finally, a significant proportion of epilepsies, particularly the milder forms, have a polygenic or nongenetic background and cannot be solved by NGS.[51]

A key strength of our work is the comprehensive NGS pipeline accounting for SNVs, CNVs, and mtDNA variants.[30] We found that more than 30% of diagnostic variants were either mitochondrial variants or CNVs, both of which are particularly relevant in the pathogenesis of epilepsies.[4,23] In our cohort, classic CNV syndromes were observed in 6 cases, predominantly in patients with NAFE (the most frequent epilepsy syndrome in our study). In addition, CNVs affecting monogenic epilepsy genes were detected in 2 cases, yielding an overall pathogenic CNV detection rate of 7.5%. This rate aligns with previous large-scale research because CNVs have been reported in 3% across all epilepsy phenotypes[6] and in 10% of a cohort with more severely affected epilepsy plus phenotypes.[4] These differences likely stem from patient selection. While the first study included a broad spectrum of common epilepsy phenotypes and the second focused on epilepsy plus, our study included all epilepsy syndromes but prioritized patients with a higher likelihood of a monogenic cause.

Both CNVs and mitochondrial mutations may be missed by many commercial gene panels. By comparing our results with simulated gene panels, we confirm previous findings that unbiased genetic testing approaches are advantageous over commercial panels.[16] Their contents vary significantly between different providers. Nonetheless, a combination of all 3 applied panels covered more than 90% of single-gene etiologies and only missed genes rarely associated with seizures.

First, *ZMYM2* has been related to seizures in few cases[52] and is part of a dual diagnosis (along with *SCN1A*) in our case, making a significant contribution to the epilepsy phenotype even more unlikely. Moreover, *NF2* is not a typical epilepsy gene, and usually, additional clinical features point toward this genetic etiology, regardless of the presence of epilepsy. However, it is noteworthy that both genes that were not covered by the panels are included in the recently published comprehensive review summarizing a total of 1,506 epilepsy-associated genes.[45]

Another advantage of this study is that our analysis was confined to a real-world adult cohort, representative of patients with epilepsy seen in a tertiary care center. Unlike larger exome-based studies, most of our patients underwent thorough phenotyping, including detailed clinical, imaging, and electrophysiologic assessments. To date, there is a significant knowledge gap regarding adults with genetic epilepsies that our study aims to bridge. By focusing on this often-neglected patient group, our findings contribute to a better understanding of the genetic background and clinical implications in adults, which significantly differ from the pediatric population.

Beyond clinical diagnostics, our unbiased approach allows for the identification of novel candidate genes that may be investigated in future studies. We nominate *CLASP1*, which is involved in microtubule stabilization and neuronal growth,[41] as an epilepsy candidate gene. We found 2 patients with heterozygous truncating variants and focal epilepsy with comorbid neurocognitive issues. In addition, 2 truncating variants were identified in the Epi25 study, which also occurred in patients with focal epilepsy, while no truncating variants were observed in nonepileptic controls.[2] Finally, biallelic missense variants in *CLASP1* have been found in a consanguineous family with lissencephaly, further supporting its involvement in epilepsy genetics.[42] The gene is also highly intolerant against loss-of-function variation, as reflected by a gnomAD pLI of 1. We are aware that these findings are hypothesis generating, and more cases as well as burden analyses are required to confirm a potential association.

A main limitation of our work is that diagnostic outcomes may be negatively affected by the low rate of duo and trio sequencing. This poses a major challenge in adult clinical care, where parents and other relatives are often unavailable for genetic testing. A broader availability of family genotyping could provide valuable insights into inheritance patterns or de novo mutagenesis, both of which are crucial for determining pathogenicity. Incorporating this information may even enhance the diagnostic yield by clarifying the pathogenic role of some reported VUS. Particularly in the absence of such data, there is a strong need to continuously improve and update

variant databases as well as in silico predictions to discern causative from benign variation. Recently, artificial intelligence–based tools such as AlphaMissense have been developed to tackle this problem.[32] To our knowledge, this work provides the first application of AlphaMissense in an epilepsy cohort with diagnostically curated missense variants. Almost all variants classified as causative were correctly predicted as pathogenic by AlphaMissense, while predictions were more heterogeneous for VUS.

In conclusion, the results from our study reinforce the application of comprehensive genetic testing strategies, which include the analysis of CNVs and mtDNA variants, in adults with epilepsy. We demonstrate that molecular diagnoses may inform clinical management in up to a quarter of solved cases. Our data confirm the superiority of unbiased genetic testing over targeted approaches. Furthermore, our study contributes relevant research findings, including expansions of known genotype-phenotype correlations and the identification of *CLASP1* as a novel candidate gene.

Author Contributions

M. Krenn: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. M. Wagner: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. K. Trimmel: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S. Bonelli: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J. Rath: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. J. Jud: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Schwarz: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. I. Milenkovic: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. R. Weng: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data. J. Koren: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. C. Baumgartner: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Brugger: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. T. Brunet: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. E. Graf: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J. Winkelmann: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. S. Aull-Watschinger: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. F. Zimprich: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. E. Pataraia: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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