

ORIGINAL ARTICLE

Rare genetic variation in adults with surgically treated temporal lobe epilepsy: An exome sequencing study

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Abstract

Objective: To determine the frequency of monogenic variants and pathogenic copy number variants (CNVs) in adults with surgically treated temporal lobe epilepsy (TLE).

Methods: We performed exome sequencing (ES), including CNV analysis, in 45 adults with TLE who had previously undergone epilepsy surgery. A diagnostic exome-wide analysis was conducted to identify (likely) pathogenic variants related to epilepsy. In an exploratory approach, we screened a curated list of 45 common epilepsy genes for rare deleterious variants based on AlphaMissense and REVEL in silico predictions.

Results: No highly penetrant monogenic variants were identified in this cohort. Pathogenic CNVs were detected in two individuals, corresponding to 4.4% of the cohort: a 16p13.11 deletion in a surgery responder and a 22q11.2 duplication in a surgery nonresponder. Both CNVs are characterized by reduced penetrance and broad phenotypic variability. Moreover, we identified two predicted deleterious missense variants in the epilepsy-associated genes *KCNT1* and *SLC2A1*, both of which were detected in surgery nonresponders. In silico analyses, including structural evidence, suggested a potential impact on protein function; however, their clinical significance remains uncertain.

Significance: Monogenic causes are exceedingly rare in adults with surgically treated TLE, whereas CNVs may contribute to disease susceptibility in a small subset of patients. Our findings are consistent with previous reports indicating a low diagnostic yield of presurgical genetic testing and further support a predominantly polygenic architecture of TLE.

Plain Language Summary: Temporal lobe epilepsy (TLE) is a common type of epilepsy in adults, and some patients undergo surgery when medications fail.

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It is unclear whether genetic testing can help predict surgical success. In this study, we used comprehensive genetic testing in 45 adult TLE patients who had undergone epilepsy surgery. We did not find single-gene causes of epilepsy. Two patients had structural genetic changes, which increase epilepsy risk but show variable effects. We also identified a few rare variants in epilepsy-related genes, but their clinical relevance remains uncertain. Overall, our results suggest that single-gene causes are rare in adults with TLE. Genetic testing may therefore have a low diagnostic rate in this population.

KEYWORDS

epilepsy, epilepsy surgery, exome sequencing, genetic testing, temporal lobe epilepsy

1 | INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in adulthood.¹ This type of epilepsy poses a particular clinical challenge, as approximately two-thirds of patients develop drug resistance.² For this subgroup, resective epilepsy surgery represents a potentially curative treatment option, resulting in long-term seizure freedom in more than 60% of cases.^{3,4} However, a substantial proportion of patients continue to experience seizures even after surgery, underscoring the strong need for reliable predictors or biomarkers of postoperative seizure outcome, which remain limited to date.⁵

The etiology of focal epilepsies is complex, with genetic factors being increasingly recognized as key contributors, ranging from common polygenic variation to rare monogenic forms.^{6–8} While genetic testing has become clinical routine for rare and syndromic epilepsies, its role in more prevalent phenotypes, such as TLE, remains to be defined.⁹ Although reports of multiplex families with familial TLE support a strong genetic basis,¹⁰ sequencing studies have largely failed to identify reproducible monogenic causes.¹¹ In contrast, polygenic risk models support a complex genetic basis, even in familial cases.¹² More recently, somatic variants have been proposed as an additional and so far underrecognized contributor to the pathogenesis of TLE.¹³

Anecdotal reports have proposed integrating molecular biomarkers into the presurgical workup, as certain etiologies, such as channelopathies and synaptopathies, appear to be associated with unfavorable postsurgical seizure outcomes.^{14–16} In contrast, several authors have argued that a channelopathy diagnosis should not automatically preclude presurgical evaluation, as carefully selected patients may still benefit from surgery.¹⁷ On the other hand, some genetic etiologies, including variants in *GATOR1*

Key points

- No monogenic variants were identified in 45 adults with surgically treated TLE.
- Pathogenic CNVs with reduced penetrance were detected in 2/45 patients.
- Rare missense variants in *KCNT1* and *SLC2A1* highlight candidate genetic contributors.
- Our findings confirm a low yield of genetic testing in TLE.

complex genes or genetically caused hypothalamic hamartomas, have been shown to respond favorably to surgical treatment.^{15,18}

Importantly, much of this evidence originates from pediatric cohorts, which are characterized by earlier disease onset, greater phenotypic variability, and a higher rate of monogenic causes.^{19–21} In contrast, classical TLE is thought to display a more complex genetic architecture.¹² As a consequence, systematic data supporting the use of genetic testing in adult patients with TLE, which is the prototypical indication for epilepsy surgery in this age group, remain scarce. One study applied a case–control burden design to confirm the role of rare genetic variation in TLE pathogenesis but failed to demonstrate an influence on postoperative seizure outcome.²² Another recently published work reported a low diagnostic yield of presurgical genetic testing in adult patients with focal epilepsy.²³

In this study, we aimed to determine the role of genetic testing in a real-world cohort of 45 adults with TLE who had undergone epilepsy surgery and demonstrated variable postoperative seizure outcomes.

2 | PATIENTS AND METHODS

2.1 | Patient selection

Patients were selected from an in-house database comprising 518 individuals with focal epilepsy who underwent resective epilepsy surgery at the Department of Neurosurgery, Medical University of Vienna. This database included patients irrespective of etiology or postoperative outcome. Of these, blood/DNA samples and informed consent for genetic testing were available for 45 patients who fulfilled the inclusion criteria and had not undergone genetic testing as part of previous research projects or clinical routine diagnostics. All of these cases were included in the study. Patient selection was therefore exhaustive.

Included subjects were clinically evaluated at the Department of Neurology, Medical University of Vienna, Austria, prior to enrollment. All patients underwent a comprehensive diagnostic assessment, including brain magnetic resonance imaging (MRI) and prolonged video EEG monitoring followed by at least one resective epilepsy surgery at our Department of Neurosurgery between 1993 and 2024.

Patients were eligible for inclusion, if they had a diagnosis of TLE, had undergone epilepsy surgery, and had available data on postoperative seizure outcome for at least 1 year following surgery. Only cases with one of the following histopathological categories were included: hippocampal sclerosis (HS), malformation of cortical development (MCD), or no causative structural abnormality. Exclusion criteria were (1) acquired structural abnormalities according to the final histopathological evaluation of resected brain tissue (e.g., tumors, vascular malformations, inflammatory/infectious, traumatic, or ischemic lesions) as a likely cause of epilepsy, and/or (2) moderate to severe intellectual disability.

2.2 | Exome sequencing pipeline

DNA was extracted from leukocytes. Singleton ES was performed at the Institute of Human Genetics (Technical University of Munich, Germany) using a Twist Human Exome 2.0 Plus Comprehensive Exome Spike-in and Mitochondrial Panel for enrichment. Sequencing was carried out on an Illumina NovaSeq6000 system (Illumina, San Diego, California). ExomeDepth and Pindel were applied for the detection of CNVs.^{24,25} Details on the used ES pipeline have been published previously.²⁶

2.3 | Diagnostic exome-wide analysis

Exomes were analyzed with the aim to identify potential monogenic variants underlying the epilepsy phenotype using a phenotype-based filter with an Online Mendelian Inheritance in Man (OMIM) full-term search. Variants were filtered based on minor allele frequency (MAF), which was estimated using our in-house database and confirmed by gnomAD (<0.1% for recessive, <0.01% for dominant filters). Variants were classified according to the standards of the American College of Medical Genetics and Genomics (ACMG).²⁷

2.4 | Exploratory gene set-based analysis

We used an exploratory approach to screen for very rare (gnomAD MAF <0.01%) consistently predicted deleterious missense variants (defined by an AlphaMissense score >0.564 and a REVEL score >0.644)^{28,29} in a curated set of 45 “common epilepsy” monogenic genes, including *HCN2*, *HCN1*, *GABRD*, *CHD4*, *RORB*, *GABRG2*, *GABRB2*, *GABRA5*, *KCNC2*, *CUX2*, *GABRA1*, *CHD2*, *CACNA1A*, *SLC6A1*, *SCN2A*, *SCN1B*, *SLC32A1*, *SCN1A*, *STX1B*, *SLC2A1*, *KCNA2*, *LGII*, *CHRNA4*, *CHRNA2*, *CHRNA2*, *VPS13A*, *PRIMA1*, *TSC1*, *TSC2*, *NPRL3*, *NPRL2*, *RELN*, *PIK3C2B*, *KCNT1*, *ARHGEF9*, *PCDH19*, *KCNQ2*, *KCNQ3*, *SYN1*, *PRRT2*, *CNKS2*, *DEPDC5*, *GRIN2A*, *TBC1D24*, and *SCN8A*, selected according to Oliver et al (“Genes4Epilepsy”).³⁰ In silico prediction scores were obtained from the Franklin by Genoox platform (<https://franklin.genoox.com>), which aggregates annotations from multiple prediction tools.

2.5 | In silico characterization

An in silico characterization for qualifying variants was performed according to the abovementioned screening criteria, using the AlphaFold Protein Structure Database for structure prediction, represented by the pLDDT score.^{31–33} AF structure models and AM hotspot models of *KCNT1* and *SLC2A1* were analyzed, accessed on April 9, 2026. Due to different splice isoforms of *KCNT1*, the sequence numbering in the AlphaFold model deviates from the reference numbering used in this publication. The Serine 963 position in the AlphaFold numbering (UniProt isoform accession: Q5JUK3–1) corresponds to the Serine 982 position in the reference numbering (UniProt isoform accession: Q5JUK3–3). Experimental models of *KCNT1* (PDB entries 8HIR and 8HKF)³⁴ and *SLC2A1* (PDB entry

6THA)³⁵ were analyzed for the structural context of the coding variants.

2.6 | Ethics approval

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 (1933/2024). Informed consent was obtained from all included patients or their legal guardians prior to study inclusion.

3 | RESULTS

3.1 | Baseline cohort characterization

The cohort consisted of 45 individuals with TLE. Approximately half of the patients (46.7%) were female. The median age at seizure onset was 11 years, ranging from 7 months to 53 years. Brain MRI revealed HS and/or hippocampal atrophy in more than 60% of the cohort. One patient had an isolated MCD, one a dual pathology (i.e., MCD and HS/HA), and one-third of the patients had no or unspecific MRI abnormalities.

All patients experienced focal impaired consciousness seizures before surgery, and 91.1% had experienced at least one bilateral tonic-clonic seizure (BTCS). More than half of the individuals reported focal preserved consciousness seizures. According to the currently used definition of the International League Against Epilepsy (ILAE),³⁶ 41 patients (91.1%) fulfilled the criteria of drug-resistant epilepsy prior to surgery.

A positive family history, defined as at least one first-degree or two second-degree relatives with seizures, was only reported in two cases (4.4%). Six patients (13.3%) had a history of febrile seizures. Neuropsychiatric comorbidities were reported in 24 patients (53.3%). However, none of the included individuals was diagnosed with autism spectrum disorder or intellectual disability. Further, no dysmorphic features were reported.

The median time from epilepsy onset to the first resective epilepsy surgery was 18 years, ranging from 1 to 50 years. Histology revealed HS in two-thirds of cases. In three patients, MCDs were found as the primary etiology, and in the remaining cases (26.7%), no specific histopathological abnormality was identified.

Almost half of the patients responded to surgery, which was defined as ILAE Class 1a (42.2%) or Class 1 (2.2%) at the last follow-up. Four patients were classified as ILAE Class 2 (8.9%), 10 as Class 3 (22.2%), 4 as Class 4 (8.9%), and 7 as Class 5 (15.6%). None of the patients

was classified as Class 6. The median follow-up time since the last resective epilepsy surgery was 10 years (range 1 to 25 years). Three patients received a vagus nerve stimulator due to ongoing seizures after resective surgery.

Baseline characteristics of all 45 included individuals are summarized in Table 1.

3.2 | Diagnostic (exome-wide) findings

Our diagnostic analysis approach yielded no pathogenic or likely pathogenic single nucleotide variants (SNVs) according to ACMG criteria, and thus no highly penetrant monogenic variants in epilepsy-related disease genes. However, pathogenic CNVs were detected in two cases, both of which are characterized by markedly reduced penetrance.³⁷

1. 16p13.11 deletion: NC_000016.9:g.(14766453_15069057)_ (16297431_17202552)del
2. 22q11.2 duplication: NC_000022.10:g.(18650803_18893888)_ (21386101_21799211)dup

3.3 | Rare missense variants in epilepsy genes

In silico prediction-based screening of 45 “common epilepsy” monogenic genes identified two rare predicted deleterious missense variants in genes previously associated with focal epilepsies:

1. *KCNT1* (NM_020822.3): c.2945C>T, p.Ser982Phe (heterozygous)
2. *SLC2A1* (NM_006516.4): c.1123T>A, p.Phe375Ile (heterozygous)

Both missense variants were classified as VUS according to ACMG criteria. The *SLC2A1* variant has already been reported as VUS in the ClinVar database (accession number: VCV003061552.2), whereas the *KCNT1* variant has not been described previously.

3.4 | Clinical vignettes

16p13.11 deletion (pathogenic CNV with reduced penetrance). This deletion was identified in a male patient with left mesial TLE who had seizure onset at preschool age with focal seizures characterized by sudden nausea, discomfort, facial flushing, fixed gaze, later accompanied by oral automatisms. Despite multiple ASM trials, seizures remained drug-resistant, with occasional progression to

TABLE 1 Demographic and clinical characteristics of all 45 patients with temporal lobe epilepsy who underwent genetic testing.

Characteristics	Total cohort n = 45 (%)
Sex (female)	21 (46.7)
Median age at seizure onset in years (range)	11 (0–53)
MRI	
HS/HA	28 (62.2)
MCD	1 (2.2)
Dual pathology (MCD + HS/HA)	1 (2.2)
MRI normal/unspecific	15 (33.3)
Seizure type before surgery	
Focal preserved consciousness seizures	27 (60)
Focal impaired consciousness seizures	45 (100)
BTCS	41 (91.1)
Positive family history (i.e., at least one first-degree or two second-degree relatives with seizures)	2 (4.4)
Cognitive abilities (based on presurgical neuropsychological assessment)	
Below average	21 (46.7)
Average	16 (35.6)
Above average	3 (6.7)
No data available	5 (11.1)
Neuropsychiatric comorbidities (any)	24 (53.3)
Invasive (phase II) evaluation performed	6 (13.3)
History of febrile seizures	6 (13.3)
Type of surgery	
SAH	25 (55.6)
ATLR	17 (37.8)
Other	3 (6.7)
Median time from epilepsy onset to (first) surgery in years (range)	18 (1–50)
Histology	
Hippocampal sclerosis	30 (66.7)
MCD	3 (6.7)
No (specific) pathology	12 (26.7)
ILAE outcome class at last follow-up	
1a	19 (42.2)
1	1 (2.2)
2	4 (8.9)
3	10 (22.2)
4	4 (8.9)
5	7 (15.6)
Median follow-up since (last) resective surgery in years (range)	10 (1–25)
Neurostimulation (VNS)	3 (6.7)

Abbreviations: ATLR, anterior temporal lobe resection; BTCS, bilateral tonic-clonic seizures; HA, hippocampal atrophy; HS, hippocampal sclerosis; MCD, malformation of cortical development; SAH, selective amygdalohippocampectomy; VNS, vagus nerve stimulation.

BTCS. Presurgical evaluation demonstrated left temporal and generalized interictal epileptiform discharges. A left mesial temporal onset was confirmed by invasive EEG. Following surgery, histology revealed mMCD with gliosis, and the patient remained seizure-free throughout 4 years of follow-up (ILAE Class 1a outcome).

22q11.2 duplication (pathogenic CNV with reduced penetrance). This CNV was detected in a female patient with right mesial TLE who developed seizures five years after a traumatic brain injury at age 21. Seizures were initially characterized by an epigastric aura, unpleasant metallic taste, nausea, and a pulling sensation throughout the body, sometimes progressing to leftward head and eye deviation, nystagmus, manual automatisms, and urinary urge, with rare evolution to BTCS. Presurgical evaluation demonstrated a right temporal seizure onset with bilateral interictal temporal spikes and right HS, which was confirmed histologically. Despite an initial reduction in seizure frequency, the patient had ongoing seizures after surgery (ILAE Class 5 outcome).

KCNT1 (VUS). This missense variant was identified in a woman who developed drug-resistant left TLE at 15 years of age with focal seizures characterized by speech disturbance, behavioral arrest, and postictal amnesia. Despite initial improvement with ASM, seizure frequency increased over time. Presurgical evaluation demonstrated concordant left temporal EEG findings and a left temporal pole encephalocele on MRI without hippocampal sclerosis. She underwent left temporal pole resection after stereo-EEG evaluation, with histopathology revealing gliosis. Although initially seizure-free, she experienced recurrent auras during follow-up (ILAE Class 2 outcome).

SLC2A1 (VUS). This missense variant was reported in a female patient who developed epilepsy with BTCS at 17 years of age without developmental delay or a family history of epilepsy. Following an initial response to levetiracetam, she progressed to pharmacoresistance with daily focal seizures characterized by an epigastric rising aura followed by impaired consciousness with partial preservation of comprehension, evolving into BTCS. Presurgical investigations demonstrated left mesial temporal seizure onset and left HS on MRI. Despite selective amygdalohippocampectomy followed by two additional temporal resections, seizures persisted during follow-up (ILAE Class 5 outcome).

Clinical and genetic details on the patients with reported variants are summarized in [Table 2](#).

3.5 | In silico analyses

According to AlphaMissense, the variants are predicted to be likely deleterious, with scores of 0.936 (*KCNT1*:

TABLE 2 Genetic and clinical details on individuals with pathogenic copy number variants (CNVs) and rare deleterious variants of uncertain significance (VUS) in “common epilepsy” monogenic genes.

ID / sex	Variant (zygosity)	Variant classification	AlphaMissense/REVEL scores	Preoperative MRI findings	Preoperative EEG findings	Type of surgery	Histological classification	Postsurgical outcome (ILAE class)	Time of follow-up after surgery (years)	Age at epilepsy onset (years)	Neuropsychiatric comorbidities	Overall cognitive abilities (neuropsychological testing)	Family history	Number of fulfilled ILAE criteria for genetic testing ^a
#2234/ male	16p13.11 deletion (heterozygous)	Pathogenic (reduced penetrance)	N.A./N.A.	Minimal hyperintensity of the left hippocampus	Left temporal onset	Left ATL	mMCD	1a	4	4	Yes (panic attacks)	Below average	Negative	1/5
#157/ female	22q11.2 duplication (heterozygous)	Pathogenic (reduced penetrance)	N.A./N.A.	Right hippocampal sclerosis and atrophy	Right temporal onset	Right ATL	Hippocampal sclerosis	5	25	26	Yes (depression)	Below average	Negative	1/5
#2303/ female	KCNT1 (NM_020822.3): c.2945C>T, p.(Ser982Phe) (heterozygous)	VUS	0.936/0.73	Tiny encephalocoele at left temporal pole	Left temporal and frontal onset	Resection of the left temporal pole, including the encephalocoele	Encephalocoele	2	3	15	Yes (depression, panic attacks)	Average	Negative	1/5
#1983/ female	SLC2A1 (NM_006516.4): c.1123T>A, p.(Phe375Ile) (heterozygous)	VUS	0.956/0.89	Left hippocampal sclerosis and atrophy	Left temporal onset	Left SAH, 2 further resections	Hippocampal sclerosis	5	6	17	None	Average	Negative	1/5

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ATL, anterior temporal lobe resection; ILAE, International League Against Epilepsy; mMCD, minimal malformation of cortical; SAH, selective amygdalohippocampectomy; VUS, variant of uncertain significance.

^aGenetic testing criteria for isolated focal epilepsy based on ILAE recommendations⁴⁶: positive family history, intellectual disability, autism, dysmorphic features, and pharmacoresistance. Column indicates the number of criteria (0–5) fulfilled by each patient. All four patients only fulfilled the pharmacoresistance criterion.

Ser982Phe) and 0.956 (*SLC2A1*: Phe375Ile), respectively. The structural role of affected amino acids is the stabilization of the local structure, rendering the positions highly intolerant to variation. *KCNT1*: Ser982Phe leads to serious steric clashes and disruption of hydrogen bonds, and *SLC2A1*: Phe375Ile disrupts the conformational changes and stability of the transport cycle. Thus, structural evidence for these variants supports the predicted deleteriousness.

In silico structural analysis using AlphaFold, AlphaMissense, and available experimental structures are provided in Figure 1.

4 | DISCUSSION

Using an exome-based approach, we found no highly penetrant monogenic variants in a well-characterized cohort of 45 subjects with TLE, while pathogenic CNVs with reduced penetrance were detected in a small subset (i.e., 4.4%) of cases. A recently published work reported a low diagnostic yield of presurgical genetic testing in adults with epilepsy.²³ Our study independently confirms these observations in an even more highly selected patient population, as we exclusively included individuals who had already undergone epilepsy surgery with long-term post-operative follow-up data available.

The aforementioned findings are also in line with emerging evidence suggesting that TLE is mainly driven by polygenic risk factors.¹² While familial aggregation and twin studies indicate a substantial heritable component,³⁸ previous sequencing studies failed to identify reproducible monogenic causes, suggesting a complex molecular background.¹¹

We identified pathogenic CNVs in two individuals, corresponding to 4.4% of the cohort, including one 16p13.11 deletion and one 22q11.2 duplication. Both detected CNVs have already been associated with a variety of neurodevelopmental phenotypes with or without seizures.^{39,40} The CNV detection rate in this study is in concordance with a previous large-scale study, which showed that 1.5%–3% of patients with common epilepsy syndromes carry pathogenic CNVs.⁴¹ Although these variants are known to contribute to disease risk, their penetrance is markedly reduced,³⁷ making their presence alone insufficient to inform clinical decision-making, including presurgical evaluation.

While we used an exome-wide approach to identify diagnostic (likely pathogenic) variants, the screen for rare missense VUS was restricted to a curated panel of 45 monogenic disease genes with well-established relevance to common forms of epilepsy, as an unbiased search would have yielded a large number of rare variants with

limited interpretability. Through this approach, we identified two VUS in *KCNT1* and *SLC2A1* that were computationally predicted to be deleterious. We acknowledge that different strategies can be applied to prioritize rare missense variants for exploratory analyses. In our study, we selected predefined thresholds based on AlphaMissense and REVEL, as recent data indicate that these represent the most accurate in silico tools for predicting the pathogenicity of epilepsy-associated missense variants.⁴²

For these two missense variants, we also performed an in silico characterization incorporating evolutionary conservation as well as structural considerations, which supported a functional relevance at the protein level (Figure 1). However, computational evidence alone is insufficient to establish pathogenicity. While TLE is not part of the classical phenotypic spectrum associated with these genes, variants in both genes have already been reported in focal epilepsies.^{43,44} Functional validation and segregation analyses are necessary to clarify their clinical relevance. While precision medicine approaches have been proposed for pathogenic variants in both *KCNT1* and *SLC2A1*,⁴⁵ our detected variants were classified as VUS and therefore did not inform clinical management. In case of a reclassification of these variants as (likely) pathogenic (e.g., based on confirming de novo mutagenesis through parental testing), we would definitely consider targeted treatment approaches.

From a clinical point of view, our results suggest that routine genetic testing may have a low diagnostic yield in carefully selected adults with TLE undergoing presurgical evaluation. However, the absence of monogenic findings in our cohort does not imply that presurgical genetic testing is generally not indicated. Recent ILAE recommendations suggest considering genetic testing in patients with isolated focal epilepsy who present with additional features such as pharmacoresistance, intellectual disability, autism, dysmorphic features, or a positive family history.⁴⁶ While all cases with qualifying variants had pharmacoresistant epilepsy, none of them had a positive family history, intellectual disability, dysmorphic, or autistic features.

It is worthy of note that the rate of ILAE Class 1 outcomes in our cohort (44%) is lower than expected. This is reflected by a previously published, overlapping mesial TLE cohort from our center, which reported long-term seizure freedom in almost 70%.⁴ For our study, only 45 out of 518 patients who underwent epilepsy surgery met the requirements of sample and consent availability as well as clinical eligibility for inclusion. The overrepresentation of refractory cases may reflect that such cases remain under follow-up care for longer periods of time, increasing the likelihood for biobanking. Seizure-free patients, by contrast, may be discharged from active follow-up earlier on.

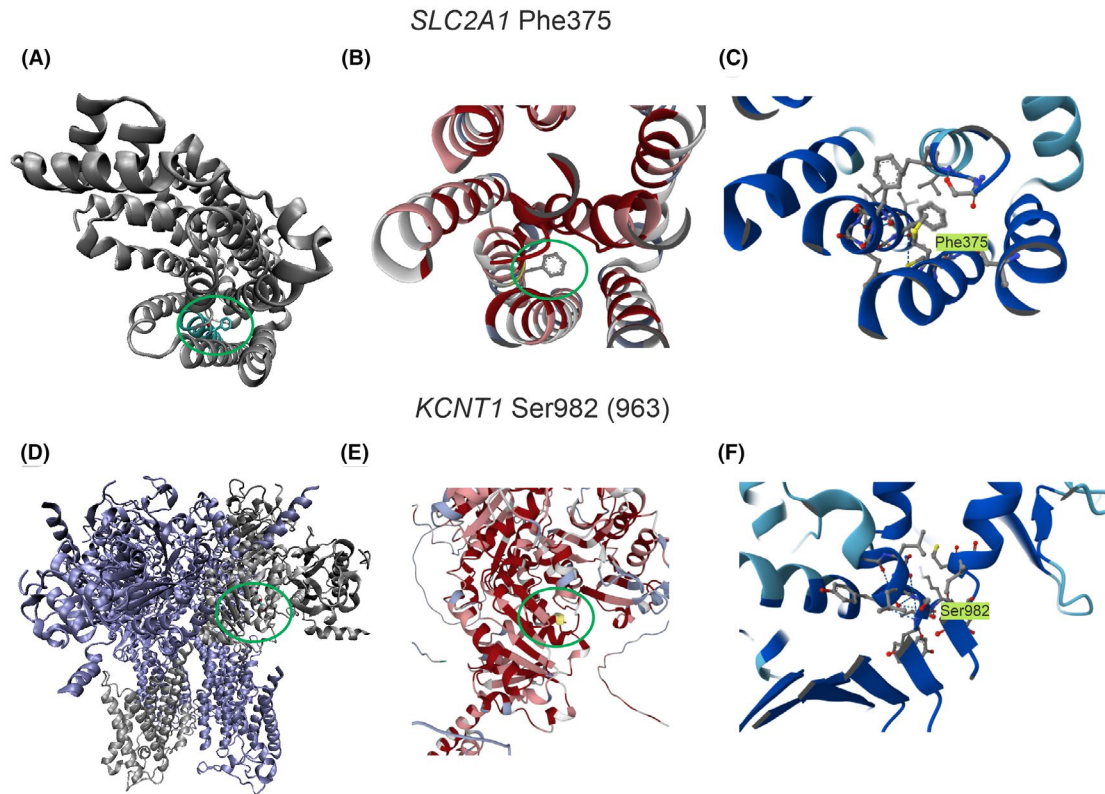


FIGURE 1 Structures of KCNT1 and SLC2A1. (A–C): SLC2A1 (glucose transporter type 1) and location of Phe375: (A) The top panel depicts the experimental structure of the transporter with PDB ID 6THA. (B) View of Phe375, localized in helix 10 which contributes to glucose transport and conformational cycling of the porter. AM model reflecting the mean predicted pathogenicity score per amino acid. (C) SLC2A1 Phe375 interactions in the AF model. (D–F): KCNT1 (sodium-activated potassium channel) and location of Ser982: (D) The entire tetrameric channel, experimental model with PDB ID 8HKF. (E) AM zoomed in model of a single subunit reflecting the mean predicted pathogenicity score per amino acid. (F) KCNT1 Ser982 in the AF model (AF and AM numbering Ser963) with the interactions of Ser982 (AF 963) highlighted. The larger Phe would lead to disruption of the structure due to steric bulk and lacking H-bonding ability. Color codes: AM models are colored with mean pathogenicity score per residue (red: likely pathogenic, blue: likely benign); experimental models: chain of interest in gray; helix 10 of the glucose transporter cyan (panel A); additional chains of the potassium channel in panel D ice blue. In the AF models, the color code represents the pLDDT score, a measure of model confidence (dark blue = very high; light blue = high; yellow = low; orange = very low). The green ellipses show the location of the variants in the overview images.

Our findings in terms of surgery outcome should therefore not be extrapolated to the general TLE population.

Our study has some limitations. First and foremost, it is limited by its small sample size and not powered to detect associations between rare variants and postoperative outcome. Large-scale studies integrating rare variant analysis, polygenic risk scores, and tissue-based sequencing approaches are required to assess the contribution of different types of genetic variation to the genetic architecture of TLE and the impact on postsurgical outcome.

Second, only singleton exomes were analyzed, and parental samples were unavailable to assess de novo variation. Although we only included cases in which histopathological evaluation did not indicate an acquired etiology, the cohort was heterogeneous and included patients with normal histology, HS, isolated gliosis, and MCDs. It is well-established that MCDs, in particular, may be caused by somatic mutations confined to brain tissue

and therefore not detectable in blood-derived DNA.⁴⁷ Such variants would have been missed by our approach.

In conclusion, our findings suggest that highly penetrant monogenic variants are exceedingly rare in adults with TLE undergoing epilepsy surgery. Consequently, routine genetic testing appears to have a low diagnostic yield in this highly selected patient population. At present, molecular findings should be interpreted with caution and require multidisciplinary case-by-case discussion as part of the presurgical assessment.

ACKNOWLEDGMENTS

Open Access funding provided by Medizinische Universität Wien.

FUNDING INFORMATION

This study was financially supported by the “Medical-Scientific Fund of the Mayor of the Federal Capital

Vienna" ("Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien").

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

The authors acknowledge the use of ChatGPT (OpenAI) and Claude (Anthropic) for language optimization during the preparation of this manuscript.

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REFERENCES

- Zarrelli MM, Beghi E, Rocca WA, Hauser WA. Incidence of epileptic syndromes in Rochester, Minnesota: 1980–1984. *Epilepsia*. 1999;40:1708–14.
- Bjorke AB, Nome CG, Falk RS, Gjerstad L, Tauboll E, Heuser K. Evaluation of long-term antiepileptic drug use in patients with temporal lobe epilepsy: assessment of risk factors for drug resistance and polypharmacy. *Seizure*. 2018;61:63–70.
- Pereira Dalio MTR, Velasco TR, Feitosa IDF, Assirati Junior JA, Carlotti Junior CG, Leite JP, et al. Long-term outcome of temporal lobe epilepsy surgery in 621 patients with hippocampal sclerosis: clinical and surgical prognostic factors. *Front Neurol*. 2022;13:833293.
- Jud J, Stefanits H, Gelpi E, Quinot V, Aull-Watschinger S, Czech T, et al. Which parameters influence cognitive, psychiatric and long-term seizure outcome in mesial temporal lobe epilepsy after selective amygdalohippocampectomy? *J Neurol*. 2024;271:4249–57.
- Cleary RA, Thompson PJ, Fox Z, Foong J. Predictors of psychiatric and seizure outcome following temporal lobe epilepsy surgery. *Epilepsia*. 2012;53:1705–12.
- International League Against Epilepsy Consortium on Complex Epilepsies. GWAS meta-analysis of over 29,000 people with epilepsy identifies 26 risk loci and subtype-specific genetic architecture. *Nat Genet*. 2023;55:1471–82.
- Epi25 collaborative. Exome sequencing of 20,979 individuals with epilepsy reveals shared and distinct ultra-rare genetic risk across disorder subtypes. *Nat Neurosci*. 2024;27:1864–79.
- Krenn M, Wagner M, Hotzy C, Graf E, Weber S, Brunet T, et al. Diagnostic exome sequencing in non-acquired focal epilepsies highlights a major role of GATOR1 complex genes. *J Med Genet*. 2020;57:624–33.
- Krenn M, Wagner M, Trimmel K, Bonelli S, Rath J, Jud J, et al. Holistic exome-based genetic testing in adults with epilepsy. *Neurol Genet*. 2025;11:e200260.
- Striano P, Gambardella A, Coppola A, Di Bonaventura C, Bovo G, Diani E, et al. Familial mesial temporal lobe epilepsy (FMTLE): a clinical and genetic study of 15 Italian families. *J Neurol*. 2008;255:16–23.
- Wong JKL, Gui H, Kwok M, Ng PW, Lui CHT, Baum L, et al. Rare variants and de novo variants in mesial temporal lobe epilepsy with hippocampal sclerosis. *Neurol Genet*. 2018;4:e245.
- Harris RV, Oliver KL, Perucca P, Striano P, Labate A, Riva A, et al. Familial mesial temporal lobe epilepsy: clinical Spectrum and genetic evidence for a polygenic architecture. *Ann Neurol*. 2023;94:825–35.
- Carton RJ, Doyle MG, Kearney H, Steward CA, Lench NJ, Rogers A, et al. Somatic variants as a cause of drug-resistant epilepsy including mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia*. 2024;65:1451–61.
- Stevelink R, Sanders MW, Tuinman MP, Brilstra EH, Koeleman BP, Jansen FE, et al. Epilepsy surgery for patients with genetic refractory epilepsy: a systematic review. *Epileptic Disord*. 2018;20:99–115.
- Bosselmann CM, San Antonio-Arce V, Schulze-Bonhage A, Fauser S, Zacher P, Mayer T, et al. Genetic testing before epilepsy surgery—an exploratory survey and case collection from German epilepsy centers. *Seizure*. 2022;95:4–10.
- Krenn M, Schloegl M, Patarai E, Gelpi E, Schröder S, Rauscher C, et al. Delineation of epileptic and neurodevelopmental phenotypes associated with variants in STX1B. *Seizure*. 2021;87:25–9.
- Marques P, Moloney PB, Ji C, Zulfiqar Ali Q, Ramesh A, Goldstein DB, et al. Do germline genetic variants influence surgical outcomes in drug-resistant epilepsy? *Epilepsy Res*. 2024;206:107425.
- Wang H, Liu W, Zhang Y, Liu Q, Cai L, Jiang Y. Seizure features and outcomes in 50 children with GATOR1 variants: a retrospective study, more favorable for epilepsy surgery. *Epilepsia Open*. 2023;8:969–79.
- Becker LL, Makridis KL, Abad-Perez AT, Thomale UW, Tietze A, Elger CE, et al. The importance of routine genetic testing in pediatric epilepsy surgery. *Epilepsia Open*. 2024;9:800–7.
- Eriksson MH, Whitaker KJ, Booth J, Piper RJ, Chari A, Martin Sanfilippo P, et al. Pediatric epilepsy surgery from 2000 to 2018: changes in referral and surgical volumes, patient characteristics, genetic testing, and postsurgical outcomes. *Epilepsia*. 2023;64:2260–73.
- Straka B, Splitkova B, Vlckova M, Tesner P, Rezacova H, Krskova L, et al. Genetic testing in children enrolled in epilepsy surgery program. A real-life study. *Eur J Paediatr Neurol*. 2023;47:80–7.
- Perucca P, Stanley K, Harris N, McIntosh A, Asadi-Pooya AA, Mikati MA, et al. Rare genetic variation and outcome of surgery

- for mesial temporal lobe epilepsy. *Ann Neurol*. 2022. <https://doi.org/10.1002/ana.26581>
23. Junemann C, Stuart A, Kaur N, Wiebe S, Jette N, Singh S, et al. Low diagnostic yield of presurgical genetic testing in adult patients with epilepsy. *Epilepsia*. 2026. <https://doi.org/10.1002/epi.70291>
 24. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*. 2012;28:2747–54.
 25. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*. 2009;25:2865–71.
 26. Schmidt A, Danyel M, Grundmann K, Brunet T, Klinkhammer H, Hsieh TC, et al. Next-generation phenotyping integrated in a national framework for patients with ultrarare disorders improves genetic diagnostics and yields new molecular findings. *Nat Genet*. 2024;56:1644–53.
 27. Richards S, Aziz N, Bale S, Bick D, das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–24.
 28. Cheng J, Novati G, Pan J, Bycroft C, Žemgulytė A, Applebaum T, et al. Accurate proteome-wide missense variant effect prediction with AlphaMissense. *Science*. 2023;381:eadg7492.
 29. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99:877–85.
 30. Oliver KL, Scheffer IE, Bennett MF, Grinton BE, Bahlo M, Berkovic SF. Genes4Epilepsy: an epilepsy gene resource. *Epilepsia*. 2023;64:1368–75.
 31. Fleming J, Magana P, Nair S, Tsenkov M, Bertoni D, Pidruchna I, et al. AlphaFold protein structure database and 3D-beacons: new data and capabilities. *J Mol Biol*. 2025;437:168967.
 32. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596:583–9.
 33. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, et al. AlphaFold protein structure database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res*. 2022;50:D439–D444.
 34. Zhang J, Liu S, Fan J, Yan R, Huang B, Zhou F, et al. Structural basis of human Slo2.2 channel gating and modulation. *Cell Rep*. 2023;42:112858.
 35. Custodio TF, Paulsen PA, Frain KM, Pedersen BP. Structural comparison of GLUT1 to GLUT3 reveal transport regulation mechanism in sugar porter family. *Life Sci Alliance*. 2021;4:4.
 36. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. *Epilepsia*. 2010;51:1069–77.
 37. Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med*. 2013;15:478–81.
 38. Dang YL, Esnault K, Fitt G, O'Brien TJ, Jackson GD, Perucca P, et al. Twins with temporal lobe epilepsy: genetic contributions to hippocampal sclerosis and other subtypes. *Brain*. 2025;148:3989–99.
 39. Ensenauer RE, Adeyinka A, Flynn HC, Michels VV, Lindor NM, Dawson DB, et al. Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. *Am J Hum Genet*. 2003;73:1027–40.
 40. Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, et al. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet*. 2010;86:707–18.
 41. Niestroj LM, Perez-Palma E, Howrigan DP, Zhou Y, Cheng F, Saarentaus E, et al. Epilepsy subtype-specific copy number burden observed in a genome-wide study of 17 458 subjects. *Brain*. 2020;143:2106–18.
 42. Montanucci L, Brunger T, Bosselmann CM, Ivaniuk A, Pérez-Palma E, Lhatoo S, et al. Evaluating novel in silico tools for accurate pathogenicity classification in epilepsy-associated genetic missense variants. *Epilepsia*. 2024;65:3655–63.
 43. Gokce-Samar Z, Lesca G, Bourgeois-Vionnet J, Isnard J, Boulogne S, Valton L, et al. Insights from stereoelectroencephalography in KCNT1-related focal epilepsy suggest a multifocal and migrating epileptogenic network. *Epilepsia*. 2025;66:e90–e97.
 44. Wolking S, Becker F, Bast T, Wiemer-Kruel A, Mayer T, Lerche H, et al. Focal epilepsy in glucose transporter type 1 (Glut1) defects: case reports and a review of literature. *J Neurol*. 2014;261:1881–6.
 45. Knowles JK, Helbig I, Metcalf CS, Lubbers LS, Isom LL, Demarest S, et al. Precision medicine for genetic epilepsy on the horizon: recent advances, present challenges, and suggestions for continued progress. *Epilepsia*. 2022;63:2461–75.
 46. Krey I, Platzer K, Esterhuizen A, Berkovic SF, Helbig I, Hildebrand MS, et al. Current practice in diagnostic genetic testing of the epilepsies. *Epileptic Disord*. 2022;24:765–86.
 47. Baldassari S, Ribierre T, Marsan E, Adle-Biassette H, Ferrand-Sorbets S, Bulteau C, et al. Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. *Acta Neuropathol*. 2019;138:885–900.

How to cite this article: Pirker AP, Ernst M, Wagner M, Bonelli SB, Trimmel K, Jud J, et al. Rare genetic variation in adults with surgically treated temporal lobe epilepsy: An exome sequencing study. *Epilepsia Open*. 2026;00:1–10. <https://doi.org/10.1002/epi.70350>