



Article

Whole-Exome Sequencing Utility and Drug Resistance in a Real-World Epilepsy Cohort from Southern Kazakhstan

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Abstract

Background/Objectives: In resource-limited regions, limited access to genetic testing may delay etiological diagnosis in selected patients with epilepsy and complex neurological manifestations, particularly when the phenotype raises suspicion of an underlying genetic etiology. This study aimed to assess the diagnostic value and clinical utility of phenotype-guided WES in selected patients aged ≥ 17 years with epilepsy and complex neurological manifestations in Southern Kazakhstan and to characterize clinical, therapeutic, and molecular features associated with drug-resistant epilepsy (DRE). **Methods:** This observational cohort included 78 patients aged ≥ 17 years with epilepsy and complex neurological manifestations identified through 25 outpatient medical centers. After enrollment, all patients underwent structured HPO-based phenotyping, assessment of anti-seizure medication exposure, seizure burden, treatment response, and proband-only WES. Variants were interpreted according to ACMG/AMP guidelines. Exploratory multivariable logistic regression was used to assess factors associated with DRE. **Results:** P/LP variants were identified in 12/78 patients, corresponding to a diagnostic yield of 15.4%. VUS were detected in 16/78 patients (20.5%). DRE was present in 41 patients (52.6%), all of whom were receiving polytherapy. Carbamazepine was the most frequently used antiseizure medication (52/78, 66.7%), followed by valproic acid (32/78, 41.0%) and levetiracetam (20/78, 25.6%). Patients with DRE had earlier seizure onset, more frequent definite structural MRI abnormalities, and a higher proportion of P/LP variants. In the adjusted model, P/LP variant presence and definite structural MRI abnormality were associated with DRE. **Conclusions:** Phenotype-guided proband-only WES provided clinically relevant diagnostic information in a real-world epilepsy cohort with complex neurological manifestations in a resource-limited outpatient setting. The observed burden of DRE and polytherapy supports the potential value of integrating therapeutic profiling, neuroimaging, and genomic testing in the etiological evaluation of patients with epilepsy and additional neurological features.



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1. Introduction

Epilepsy is a heterogeneous neurological condition with diverse etiologies, including structural, genetic, metabolic, immune, infectious, and unknown causes. In routine clinical practice, etiological clarification is particularly important for patients with difficult-to-treat epilepsy, early disease onset, cognitive impairment, structural brain abnormalities, or other complex neurological features that collectively indicate clinically complex epilepsy phenotype. Among these patients, drug-resistant epilepsy (DRE) represents a major clinical challenge and is defined by the International League Against Epilepsy (ILAE) as failure to achieve sustained seizure freedom despite adequate trials of at least two appropriately selected and tolerated antiseizure medications [1]. This definition requires accurate seizure classification, appropriate medication selection, sufficient dosing, adequate treatment duration, and assessment of treatment adherence to distinguish true pharmacoresistance from pseudo-resistance related to diagnostic or therapeutic factors [2,3].

Once pseudo-resistance has been excluded, true DRE may reflect underlying structural, biological, or genetic mechanisms. Structurally determined epilepsies, particularly those associated with malformations of cortical development, may exhibit intrinsic resistance due to stable epileptogenic substrates [4]. These observations support the concept that, in a subset of patients, DRE may reflect an underlying biological or etiological substrate rather than simply a late consequence of prolonged epilepsy [5]. DRE affects approximately one-third of individuals with epilepsy despite the availability of multiple antiseizure medications [6,7]. Pharmacoresistance is associated with persistent seizures, increased risks of injury and sudden unexpected death in epilepsy, cognitive and psychiatric comorbidities, reduced quality of life, hospitalization, and long-term disability [7–10]. The burden of DRE is particularly high among patients with early-onset epilepsy, developmental or cognitive impairment, structural brain abnormalities, or suspected genetic etiologies [8–10].

Genetic factors constitute an important etiological component of epilepsy, particularly in patients with early-onset seizures, developmental and epileptic encephalopathies, intellectual disability, structural brain abnormalities, and complex neurological phenotypes. Pathogenic variants affecting ion channels, synaptic proteins, and regulators of cortical development may contribute to epileptogenesis and variability in treatment response [11–14]. In this context, drug resistance may reflect, at least in a subset of patients, the underlying molecular and structural etiology rather than suboptimal antiseizure therapy alone. Therapeutic profiling provides clinical context for interpreting genetic findings, as antiseizure medication patterns, polytherapy, seizure burden, and treatment response may help distinguish pharmacological complexity from underlying etiological complexity, especially in real-world settings where access to specialized epilepsy care and genetic testing may be limited.

The implementation of next-generation sequencing (NGS) has substantially changed the diagnostic evaluation of epilepsy. In clinically selected patients, particularly those with early-onset disease, neurodevelopmental comorbidities, structural abnormalities, or treatment resistance, NGS-based approaches, including multigene panels and whole-exome sequencing (WES), can identify clinically relevant molecular genetic variations [15,16]. WES is especially useful when the phenotype is heterogeneous or when targeted testing is unlikely to capture the full spectrum of relevant genetic etiologies. Identification of

pathogenic or likely pathogenic variants may refine etiological classification, inform prognosis, guide genetic counselling, and, in selected cases, influence treatment or surveillance strategies [17,18].

However, the clinical utility of WES in clinically selected real-world epilepsy cohorts remains incompletely defined, particularly in resource-limited and underrepresented regions. In routine outpatient practice, patients identified for etiological clarification may have longstanding disease, incomplete early-life documentation, heterogeneous seizure histories, variable exposure to antiseizure medications, and mixed structural, genetic, or unknown etiologies. These factors may complicate both variant interpretation and assessment of diagnostic value [15–18]. Despite advances in epilepsy genomics, patients from Central Asia remain underrepresented in studies of genetic testing in epilepsy [17,19]. In Kazakhstan and neighboring countries, access to comprehensive genetic testing remains heterogeneous and constrained by infrastructural, financial, and organizational factors [20,21]. National epidemiological data further indicate a substantial burden of epilepsy in Kazakhstan [22]. Therefore, this study aimed to assess the diagnostic value and clinical utility of phenotype-guided proband-only WES in selected patients with epilepsy and complex neurological manifestations identified through outpatient neurology practice in Southern Kazakhstan. We further aimed to characterize clinical, therapeutic, neuroimaging, and molecular profiles of the cohort, compare findings between drug-resistant and drug-responsive epilepsy, and explore factors associated with DRE in this real-world resource-limited setting.

2. Materials and Methods

2.1. Study Design and Setting

This study was conducted as an observational cohort study with prospective patient enrollment and retrospective abstraction of clinical, neuroimaging, electroencephalographic, and treatment-response data. The study evaluated the diagnostic value and clinical utility of whole-exome sequencing (WES) in patients with epilepsy and complex neurological manifestations identified through routine outpatient neurology practice in Southern Kazakhstan. Patient enrollment was carried out from March 2024 to June 2025 across 25 outpatient medical centers providing routine epilepsy care. All included patients underwent standardized clinical phenotyping and proband-only WES. Following genetic testing, structured reverse phenotyping was performed to support diagnostic interpretation and assess concordance between molecular findings and clinical features.

2.2. Study Population

The study population included 78 patients with epilepsy who met predefined eligibility criteria and were consecutively enrolled during the recruitment period. Participants were recruited from routine outpatient neurology practice across local polyclinics and outpatient medical centers in Southern Kazakhstan after initial identification by their treating neurologists.

Patients were eligible for inclusion if they were aged ≥ 17 years at enrollment, had an established clinical diagnosis of epilepsy, a disease duration of at least one year, and a documented history of epileptic seizures confirmed by medical records and clinical assessment. Patients were included only if sufficient clinical information was available to characterize seizure history and assess treatment response.

Patients were excluded if they had psychogenic non-epileptic seizures, acute symptomatic seizures, or seizures clearly attributable to acquired etiologies, including traumatic brain injury, stroke or other vascular brain injury, central nervous system infection, brain tumor, or neurodegenerative/dementing disorder. Patients were also excluded in cases of documented poor adherence to antiseizure medication therapy, inadequate antiseizure

medication dosing, progressive infectious or metabolic encephalopathies, or insufficient clinical data for reliable assessment of seizure characteristics or treatment response.

2.3. Stratification According to Treatment Response

After inclusion, patients were stratified according to treatment response into drug-resistant and drug-responsive epilepsy groups. Treatment response was determined through review of medical records, antiseizure medication history, documented clinical outcomes, and structured patient interviews. The assessment included seizure history, medication selection, dosing adequacy, treatment duration, tolerability, and evidence of sustained seizure freedom.

DRE was defined according to the ILAE consensus definition as failure to achieve sustained seizure freedom despite adequate trials of at least two appropriately selected and tolerated antiseizure medications, administered either as monotherapy or in combination [1,23]. Sustained seizure freedom was defined as a minimum of 12 months without seizures. Patients who did not meet ILAE criteria for DRE and had achieved sustained seizure freedom on their current antiseizure medication regimen were classified as having drug-responsive epilepsy.

Therapeutic data were collected from medical records and structured patient interviews. Extracted variables included current antiseizure medication regimen, mono- or polytherapy status, seizure frequency, history of seizure clusters or status epilepticus, postictal confusion, treatment duration, tolerability, and documented treatment response. Polytherapy was defined as the concomitant use of two or more antiseizure medications at the time of assessment. In the present cohort, all patients classified as having DRE according to ILAE criteria were receiving polytherapy. Antiseizure medication data were analyzed descriptively and were used to characterize the real-world therapeutic profile of the cohort rather than to compare the efficacy of individual medications. Polytherapy was recorded as a therapeutic characteristic and was not used as the sole criterion for defining DRE.

2.4. Deep Clinical Phenotyping

Deep clinical phenotyping was performed in all enrolled patients ($n = 78$) using a standardized approach based on the Human Phenotype Ontology (HPO) framework [24]. Phenotypic data were collected systematically to characterize the clinical spectrum of epilepsy and to support subsequent interpretation of WES findings.

Clinical information was obtained through structured clinical assessment and review of available medical records. Collected variables included age at epilepsy onset, seizure semiology and classification, disease course, antiseizure medication history, treatment response, reported adverse effects, neurological examination findings, motor abnormalities, cognitive and behavioral features, and relevant systemic manifestations when present.

EEG and brain MRI data were incorporated into the phenotypic dataset based on available clinical documentation. These studies had been performed as part of routine clinical care before study inclusion. Because data were obtained from multiple outpatient centers, EEG and MRI acquisition protocols were not standardized across sites, and the use of epilepsy-specific MRI protocols could not be consistently confirmed.

Phenotypic features were manually encoded using standardized HPO terms. HPO annotation included seizure-related features, neurological and developmental findings, motor abnormalities, cognitive and behavioral features, and additional system-level manifestations. Initial phenotyping was performed by three clinicians with expertise in neurology. To ensure consistency, annotations were independently reviewed by two additional investigators, and discrepancies were resolved by consensus with final specialist review.

The resulting structured phenotypic dataset was used to describe the cohort, support WES interpretation, and guide post hoc reverse phenotyping after identification of candidate genetic variants.

2.5. Genetic Testing: WES

WES was performed in all enrolled patients ($n = 78$) using a proband-only approach. Sequencing was conducted at 3Billion Inc. (Seoul, Republic of Korea) using the Illumina NovaSeq X platform (Illumina, Inc., San Diego, CA, USA), with exome capture based on the xGen Exome Research Panel v2. The reported mean target coverage was approximately $172\times$, with 99.4% of target bases covered at a depth of $\geq 20\times$.

Bioinformatic processing and variant calling were performed using the laboratory's validated analysis pipeline, which enabled detection of single-nucleotide variants and small insertions/deletions. Variant interpretation was performed using EVIDENCE software, version 5.1 (3Billion Inc., Seoul, Republic of Korea) according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines [25,26]. Variants were classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS). Pathogenic and likely pathogenic variants were considered diagnostically relevant and were used to determine the molecular diagnostic yield. VUS were reported separately and were not considered definitive molecular diagnoses.

Segregation analysis was performed when biological samples from parents or other available family members could be obtained. Sequence variants were validated using Sanger sequencing at Genomed, Almaty, Republic of Kazakhstan. Chromosomal microarray analysis (CMA; GENOSCAN 3000 genetic analyzer, Genomed, Almaty, Kazakhstan) was performed in selected cases when WES suggested large deletions, duplications, or other copy number alterations. In these cases, CMA was used to confirm and further characterize CNVs detected by exome-based analysis. Segregation results were used to evaluate inheritance patterns and support diagnostic interpretation. A comprehensive overview of the study workflow is presented in Figure 1.

2.6. Statistical Analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were assessed for distribution characteristics and are presented as mean $\pm$ standard deviation (SD) or median and interquartile range (IQR), as appropriate. Categorical variables are presented as absolute counts and percentages. Between-group comparisons according to treatment response were performed using the Mann–Whitney U test for continuous variables and the chi-square (χ^2) test for categorical variables. Fisher's exact test was applied when expected cell counts were less than 5. For categorical variables with more than two levels, global comparisons were performed using the χ^2 test. Effect sizes for categorical comparisons were reported as odds ratios (ORs) with 95% confidence intervals (CIs), where appropriate.

An exploratory multivariable binary logistic regression model was constructed to assess factors associated with drug-resistant epilepsy. DRE status was used as the dependent variable and coded as 1 for DRE and 0 for drug-responsive epilepsy. Candidate predictors were selected a priori based on clinical relevance and included age at seizure onset, presence of a pathogenic or likely pathogenic variant, MRI abnormality, and cognitive impairment. Variants of uncertain significance were not considered diagnostically confirmed variants and were not included in the pathogenic/likely pathogenic variant category. Results were reported as adjusted odds ratios (aORs) with 95% CIs and p -values. Given the limited sample size and number of predictors, the model was interpreted as exploratory.

Model fit and discrimination were assessed using the likelihood ratio test, Hosmer–Lemeshow goodness-of-fit test, Nagelkerke R^2 , classification accuracy, and the area under the receiver operating characteristic curve (AUC). All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant. Graphical representations, including the logistic regression forest plot, were generated using GraphPad Prism version 8.0.2.263 and Python version 3.1.5.

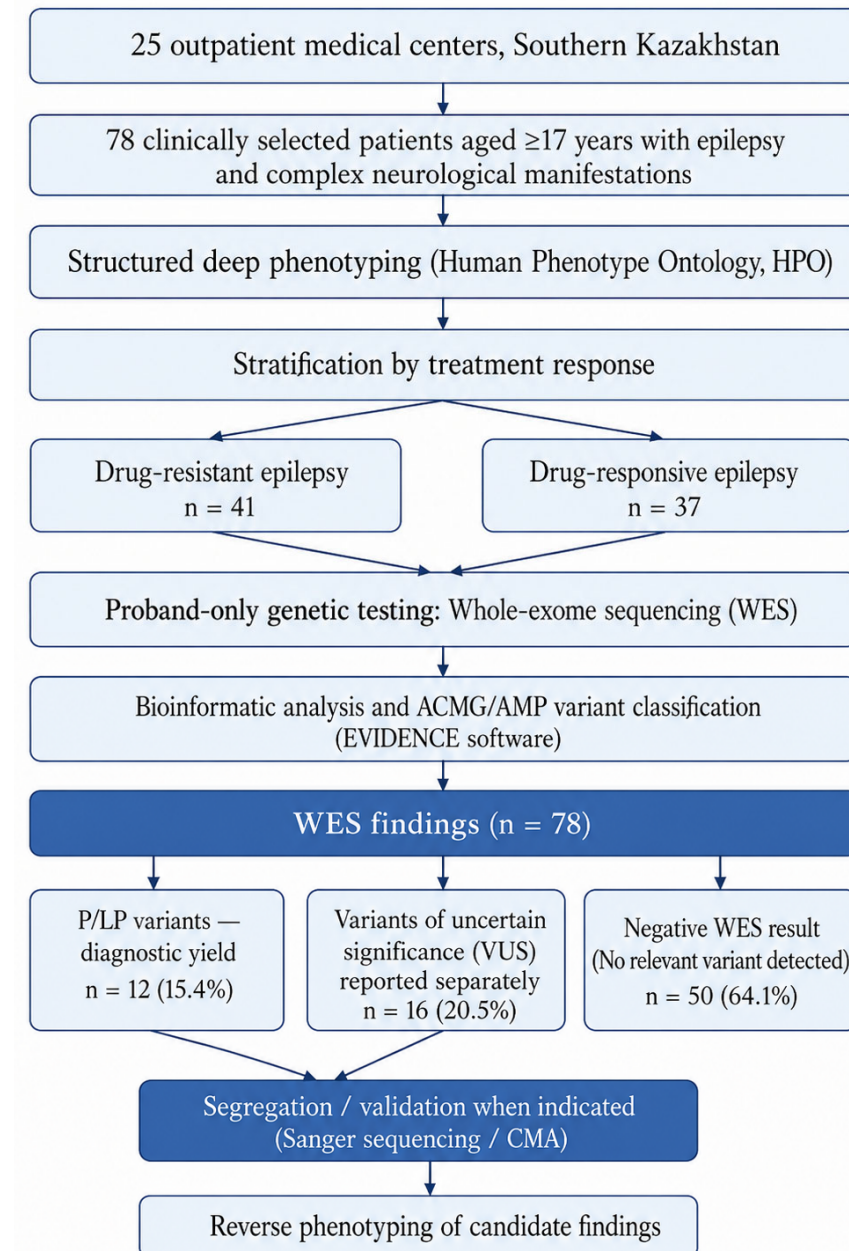


Figure 1. Study design, phenotyping, and whole-exome sequencing workflow.

2.7. Ethical Considerations

The study protocol was approved by the Local Bioethics Committee of Khoja Akhmet Yassawi International Kazakh–Turkish University (Protocol No. 21, approved on 14 November 2023; continuation approval Protocol No. 1, approved on 13 February 2026). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment and genetic testing.

3. Results

3.1. Demographic Characteristics of the Study Cohort

A total of 78 patients with epilepsy and complex neurological manifestations were included according to predefined inclusion and exclusion criteria. Participants were recruited from outpatient neurology services across 25 outpatient medical centers in Southern Kazakhstan, reflecting routine clinical practice in the region.

The median age at inclusion was 31.3 years (range, 17–63 years). The cohort included 43 females (55.1%) and 35 males (44.9%). The mean age at seizure onset was 11.9 years, with a wide range from 0.02 years, corresponding to approximately one week of life, to 57 years. Individual-level age at seizure onset for each patient is provided in Supplementary Table S1.

A positive family history of epilepsy in first-degree relatives was documented in 40 patients (51.3%), including affected siblings in 26 cases and affected parents in 14 cases. No family history was reported in 38 patients (48.7%). According to ILAE criteria, 41 patients (52.6%) met the definition of drug-resistant epilepsy, whereas 37 patients (47.4%) achieved sustained seizure freedom and were classified as having drug-responsive epilepsy.

3.2. Phenotypic Characteristics Based on Deep Clinical Phenotyping

Deep clinical phenotyping was performed in all 78 patients and demonstrated substantial clinical heterogeneity. Seizure types were classified according to the ILAE seizure classification framework. Generalized seizure phenotypes predominated and were observed in 70 patients (89.7%), with generalized tonic–clonic seizures representing the most frequent presentation. Focal seizures were identified in 2 patients (2.6%), myoclonic seizures in 3 patients (3.8%), and mixed seizure phenotypes in 3 patients (3.8%).

Beyond seizure semiology, HPO-based phenotyping demonstrated a high burden of neurodevelopmental and systemic manifestations. Cognitive impairment of any degree was documented in 60 patients (76.9%), including severe intellectual disability in 23 patients (29.5%) and mild intellectual disability in 37 patients (47.4%). A history of developmental delay and/or psychomotor retardation was reported in 8 patients (10.3%).

Motor abnormalities, including gait disturbance, spasticity, tone abnormalities, ataxia, or impaired coordination, were present in 19 patients (24.4%). Speech and language impairment was observed in 14 patients (17.9%), while visual abnormalities, including nystagmus or reduced visual acuity, were reported in 22 patients (28.2%). Behavioral and neuropsychiatric symptoms were documented in 65 patients (83.3%), most commonly including sleep disturbances, irritability, and autism spectrum–related features.

EEG abnormalities were documented in all patients. Focal epileptiform discharges were identified in 30 patients (38.5%), multifocal epileptiform activity in 21 patients (26.9%), and generalized epileptiform discharges in 18 patients (23.1%). Nonspecific background abnormalities were observed in 8 patients (10.3%), and a hypsarrhythmic pattern was documented in one patient (1.3%).

Brain MRI demonstrated definite structural abnormalities in 50 patients (64.1%), whereas 28 patients (35.9%) had normal MRI or nonspecific findings not classified as definite structural abnormalities. The most frequent definite MRI findings were cerebral atrophy or volumetric changes in 19 patients (24.4%), white matter abnormalities in 9 patients (11.5%), malformations of cortical development in 9 patients (11.5%), and destructive or cystic lesions in 5 patients (6.4%). Nonspecific MRI findings were documented separately and were not included in the binary MRI abnormality variable used for regression analysis. Comprehensive phenotypic data are presented in Supplementary Table S1.

3.3. Therapeutic Profile and Treatment Response

Therapeutic data were available for all 78 patients. According to ILAE criteria, 41 patients (52.6%) met the definition of drug-resistant epilepsy, whereas 37 patients (47.4%) were classified as drug-responsive. All patients with DRE were receiving polytherapy, while drug-responsive patients were managed with monotherapy (Table 1).

Table 1. Therapeutic profile of the study cohort.

Variable	Total Cohort, n = 78
DRE by ILAE criteria	41 (52.6%)
Drug-responsive epilepsy	37 (47.4%)
Polytherapy	41 (52.6%)
Monotherapy	37 (47.4%)
Carbamazepine	52 (66.7%)
Valproic acid	32 (41.0%)
Levetiracetam	20 (25.6%)
Lamotrigine	7 (9.0%)
Topiramate	4 (5.1%)
Oxcarbazepine	2 (2.6%)
Phenobarbital	2 (2.6%)
Clonazepam	1 (1.3%)

Carbamazepine was the most frequently used antiseizure medication and was prescribed in 52 patients (66.7%), followed by valproic acid in 32 patients (41.0%), levetiracetam in 20 patients (25.6%), lamotrigine in 7 patients (9.0%), topiramate in 4 patients (5.1%), oxcarbazepine in 2 patients (2.6%), phenobarbital in 2 patients (2.6%), and clonazepam in 1 patient (1.3%). Because patients receiving polytherapy could be counted under more than one medication category, antiseizure medication frequencies were not mutually exclusive.

3.4. Genetic Findings Identified by WES

WES was successfully performed in all 78 enrolled patients using a proband-only approach. Genetic findings were interpreted according to ACMG/AMP criteria. Pathogenic or likely pathogenic (P/LP) variants were identified in 12 patients (15.4%) and were considered diagnostically relevant. Variants of uncertain significance (VUS) were identified in 16 patients (20.5%) and were reported separately as findings requiring further validation. No relevant variants were detected in 50 patients (64.1%). The distribution of WES findings is shown in Figure 2A.

Among patients with P/LP or VUS findings, single-nucleotide variants (SNVs) were the most frequent variant type and were identified in 19 patients (67.9%). Small insertions/deletions were detected in 5 patients (17.9%), copy number variants (CNVs) in 3 patients (10.7%), and one mitochondrial DNA variant in 1 patient (3.6%). The distribution of variant types is shown in Figure 2B.

The identified variants included missense, nonsense, frameshift, splice-site variants, multi-exonic deletions, and one mitochondrial DNA variant. Loss-of-function variants, including nonsense, frameshift, canonical splice-site variants, and exon-level deletions, were observed in a subset of cases. Zygosity patterns included heterozygous, hemizygous, homozygous, compound heterozygous, mosaic, and homoplasmic variants, consistent with

autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance models (Table 2).

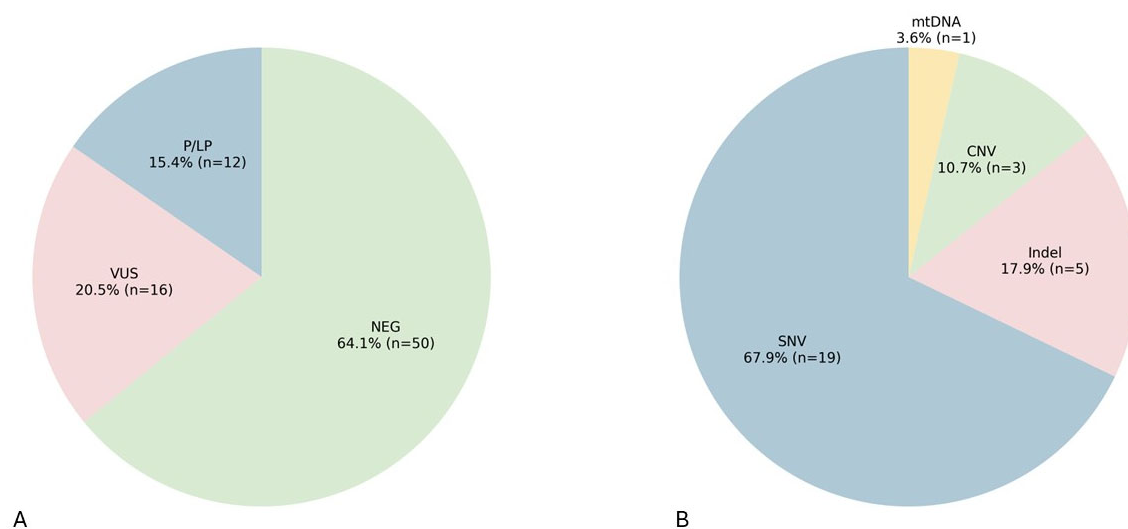


Figure 2. Distribution of whole-exome sequencing findings and variant types. **(A).** Distribution of WES findings in the total cohort. Diagnostic yield was defined as the proportion of patients with pathogenic or likely pathogenic variants. Variants of uncertain significance were reported separately and were not considered definitive molecular diagnoses. **(B).** Distribution of variant types among patients with P/LP or VUS findings. SNV, single-nucleotide variant; indel, small insertion/deletion; CNV, copy number variant; mtDNA, mitochondrial DNA variant; P/LP, pathogenic or likely pathogenic; VUS, variant of uncertain significance; NEG, negative WES result.

Table 2. Molecular findings identified in the study cohort. AD, autosomal dominant; AR, autosomal recessive; NA, not available; ILAE, International League Against Epilepsy; VUS, variant of uncertain significance; P, pathogenic; LP, likely pathogenic.

Case	Gene	Variant	Phenotype	Classification (ACMG/AMP)	Variant Inheritance	Zygosity	Inheritance Pattern	Pharmaco-resistance (ILAE Criteria)
1	<i>SLC9A6</i>	c.169+2C>T	Intellectual developmental disorder, X-linked syndromic, Christianson type	VUS	Maternal	Hemizygous	X-linked	Yes
6	<i>ATP1A3</i>	c.1969G>A	Developmental and epileptic encephalopathy 99	VUS	De novo	Heterozygous	AD	Yes
10	<i>PLP1</i>	c.742G>A	Pelizaeus-Merzbacher disease	VUS	Paternal	Hemizygous	X-linked	Yes
11	<i>COL4A1</i>	c.1292T>C	Brain small vessel disease 1 with or without ocular anomalies; BSVD1	VUS	De novo	Heterozygous	AD	Yes
18	<i>CHRNA2</i>	c.423G>A	Epilepsy, nocturnal frontal lobe, 4; ENFL4	VUS	NA	Heterozygous	AD	Yes
19	<i>GABRG2</i>	c.245G>A	Febrile seizures, familial	LP	NA	Heterozygous	AD	Yes
26	<i>GNB2</i>	c.838C>A	Neurodevelopmental disorder with hypotonia and dysmorphic facies	VUS	NA	Heterozygous	AD	Yes
27	<i>ABCD1</i>	c.455G>A	Adrenoleukodystrophy	LP	NA	Hemizygous	X-linked	Yes

Table 2. Cont.

Case	Gene	Variant	Phenotype	Classification (ACMG/AMP)	Variant Inheritance	Zygosity	Inheritance Pattern	Pharmacoresistance (ILAE Criteria)
28	<i>PHACTR1</i>	c.313C>G	Developmental and epileptic encephalopathy 70	VUS	Maternal	Heterozygous	AD	Yes
29	<i>HCN1</i>	c.539_540dup	Developmental and epileptic encephalopathy 24	VUS	De novo	Heterozygous	AD	Yes
34	<i>RFX4</i>	c.790T>C	RFX4-related intellectual disability	VUS	De novo	Heterozygous	NA	Yes
41	<i>KDM6B</i>	c.3879+25C>T	Stolerman neurodevelopmental syndrome	VUS	Paternal	Heterozygous	AD	Yes
42	<i>MAP2K2</i>	c.377A>G	Cardiofaciocutaneous syndrome 4	VUS	Paternal	Heterozygous	AD	Yes
43	<i>MT-ND6</i>	m.14484T>C	Leber optic atrophy and dystonia	P	NA	Homoplasmic		Yes
44	<i>AGA</i>	c.623-1G>A	Aspartylglucosaminuria	P	NA	Homozygous	AR	Yes
48	<i>ANKRD11</i>	c.3319_3322del	KBG syndrome	P	De novo	Heterozygous	AD	Yes
49	<i>NOTCH3</i>	c.2689G>T	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy 1	VUS	Paternal	Heterozygous	AD	Yes
54	<i>SIK1</i>	c.207G>T	Developmental and epileptic encephalopathy 30	VUS	Maternal	Heterozygous	AD	No
68	<i>PCDH19</i>	c.1031C>G	Developmental and epileptic encephalopathy 9	P	NA	mosaic	X-linked	No
69	<i>SCN1A</i>	c.266C>A	Generalized epilepsy with febrile seizures plus, type 2	VUS	De novo	Heterozygous	AD	No
71	<i>GRIA2</i>	c.835A>G	Neurodevelopmental disorder with language impairment and behavioral abnormalities	VUS	Paternal	Heterozygous	AD	Yes
72	<i>LAMC3</i>	c.3214del	Cortical malformations, occipital	P	Biparental	Homozygous	AR	Yes
73	<i>KRIT1</i>	c.1630dup	Cavernous malformations of CNS and retina	LP	De novo	Heterozygous	AD	Yes
74	<i>KCNQ5</i>	c.2162del	Intellectual developmental disorder, autosomal dominant 46	VUS	Maternal	Heterozygous	AD	Yes
75	<i>SCN1A</i>	c.554del	Dravet syndrome	LP	De novo	Heterozygous	AD	Yes

A detailed list of identified variants, affected genes, variant types, and ACMG/AMP classifications is provided in Supplementary Table S2. CNVs identified in the cohort are summarized separately in Table 3.

Overall, 9 variants were confirmed as de novo, 6 were maternally inherited, 5 were paternally inherited, and 1 demonstrated biparental transmission. In 7 cases, complete segregation assessment could not be established because parental samples were unavailable; in some instances, testing was performed in siblings rather than both parents.

Table 3. Copy number variants (CNVs) identified in the study cohort. P—pathogenic; AD—autosomal dominant; CNV—copy number variant; ILAE—International League Against Epilepsy; ACMG/AMP—American College of Medical Genetics and Genomics/Association for Molecular Pathology.

Case	Gene	Position	Phenotype	Classification (ACMG/AMP)	Variant Inheritance	Zygosity	Inheritance Pattern
5	microdeletion 15q11.2	15q11.2	15q11.2 Deletion Syndrome	P	De novo	Heterozygous	AD
33	IL1RAPL1	Xp21.3p21.2	Intellectual developmental disorder, X-linked 21	P	Maternal	Hemizygous	X-linked
76	16q24.3 deletion	16q24.3	KBG syndrome	P	Maternal	Heterozygous	AD

Reverse phenotyping was performed for patients with P/LP or VUS findings to assess concordance between molecular findings and clinical features. In patients with P/LP variants, the observed phenotypes were consistent with the corresponding gene-associated disease spectrum. VUS were interpreted cautiously and were considered findings of uncertain significance requiring further evidence rather than definitive molecular diagnoses.

3.5. Clinical, Neuroimaging, and Genetic Features Associated with Drug-Resistant Epilepsy

To identify features associated with drug-resistant epilepsy, patients were stratified into DRE (n = 41) and drug-responsive epilepsy (n = 37) groups according to ILAE criteria (Table 4).

Table 4. Comparison of clinical, neuroimaging, EEG, and molecular characteristics according to treatment response. MRI abnormality was defined as the presence of definite structural neuroimaging abnormalities. Nonspecific MRI findings were documented separately and were not included in the binary MRI abnormality variable. EEG categories were summarized descriptively. “Other EEG abnormalities” included nonspecific background changes, slow background activity, and hypsarrhythmia. *p*-values were calculated using the Mann–Whitney U test for continuous variables and the χ^2 or Fisher’s exact test for categorical variables, as appropriate. DRE, drug-resistant epilepsy; P/LP, pathogenic or likely pathogenic; VUS, variant of uncertain significance; NEG, negative WES result; IQR, interquartile range.

Variable	DRE (n = 41)	Drug-Responsive (n = 37)	<i>p</i> -Value
Age (years), median (IQR)	25 (17–35)	34 (25–39)	0.027
Sex, male, n (%)	22 (53.7)	13 (35.1)	0.157
Age at seizure onset (years), median (IQR)	4 (1–13)	12 (3–19)	0.0117
Epilepsy duration (years), median (IQR)	20 (15–24)	17 (12–28)	0.4228
Cognitive impairment, n (%)	35 (85.4)	25 (67.6)	0.111
Motor abnormalities, n (%)	15 (36.6)	14 (37.8)	1.000
Behavioral/neuropsychiatric features, n (%)	35 (85.4)	25 (67.6)	0.111
MRI abnormal, n (%)	34 (82.9)	16 (43.2)	<0.001
Focal epileptiform activity, n (%)	14 (34.1)	16 (43.2)	—
Generalized epileptiform activity, n (%)	10 (24.4)	8 (21.6)	—
Multifocal epileptiform activity, n (%)	11 (26.8)	10 (27.0)	—
Other EEG abnormalities, n (%)	6 (14.6)	3 (8.1)	—
WES findings			<0.001
—P/LP	11 (26.8)	1 (2.7)	
—VUS	14 (34.1)	2 (5.4)	
—NEG	16 (39.0)	34 (91.9)	

Clinical, neuroimaging, EEG, and molecular characteristics according to treatment response are summarized in Table 4. Patients with DRE were younger at inclusion and had earlier seizure onset than drug-responsive patients. Definite structural MRI abnormalities were substantially more frequent in the DRE group. The distribution of WES findings also differed significantly between groups, with P/LP variants enriched among patients with DRE and negative WES results predominating among drug-responsive patients.

Age at seizure onset and epilepsy duration according to treatment response are shown in Figure 3A,B. Patients with DRE had significantly earlier seizure onset than drug-responsive patients: median 4.0 years (IQR, 0.67–13.0) versus 12.0 years (IQR, 3.0–19.0), respectively ($p = 0.0117$). Epilepsy duration did not differ significantly between groups: median 20.0 years (IQR, 15.0–24.0) in the DRE group versus 17.0 years (IQR, 12.0–28.0) in the drug-responsive group ($p = 0.4228$).

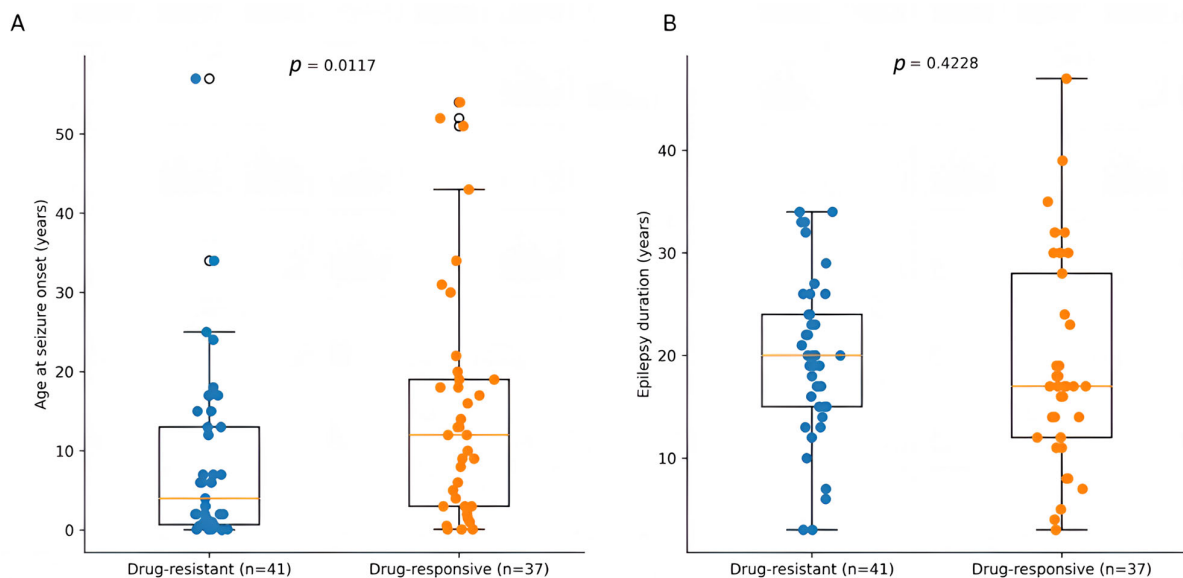


Figure 3. Age at seizure onset and epilepsy duration according to treatment response. (A) Age at seizure onset was significantly earlier in patients with DRE compared with drug-responsive patients. (B) Epilepsy duration did not differ significantly between groups. Data are shown as median and interquartile range. Each dot represents an individual patient. DRE, drug-resistant epilepsy.

An exploratory multivariable binary logistic regression model was then constructed with DRE status as the dependent variable. The model included age at seizure onset, presence of a P/LP variant, MRI abnormality, and cognitive impairment as predefined predictors. In the adjusted model, the presence of a P/LP variant was associated with DRE (aOR, 11.19; 95% CI, 1.17–107.33; $p = 0.036$), as was definite structural MRI abnormality (aOR, 6.29; 95% CI, 1.95–20.34; $p = 0.002$). Age at seizure onset (aOR, 0.97 per year; 95% CI, 0.94–1.01; $p = 0.152$) and cognitive impairment (aOR, 1.44; 95% CI, 0.40–5.15; $p = 0.571$) were not significantly associated with DRE after adjustment. Adjusted odds ratios are shown in Figure 4 and detailed in Table 5.

The overall logistic regression model was statistically significant by likelihood ratio test, $\chi^2(4) = 25.71$, $p < 0.001$. The Hosmer–Lemeshow test did not indicate poor fit ($p = 0.515$). Nagelkerke R^2 was 0.375, classification accuracy was 73.1%, and AUC was 0.811.

Table 5. Exploratory multivariable logistic regression model for drug-resistant epilepsy. DRE was used as the dependent variable. Predictors were selected a priori based on clinical relevance. DRE, drug-resistant epilepsy; P/LP, pathogenic or likely pathogenic; aOR, adjusted odds ratio; CI, confidence interval.

Predictor	β Coefficient	SE	aOR, Exp (B)	95% CI	p-Value
Age at seizure onset, per year	−0.027	0.019	0.97	0.94–1.01	0.152
P/LP variant present	2.415	1.153	11.19	1.17–107.33	0.036
Definite structural MRI abnormality	1.839	0.599	6.29	1.95–20.34	0.002
Cognitive impairment	0.367	0.649	1.44	0.40–5.15	0.571

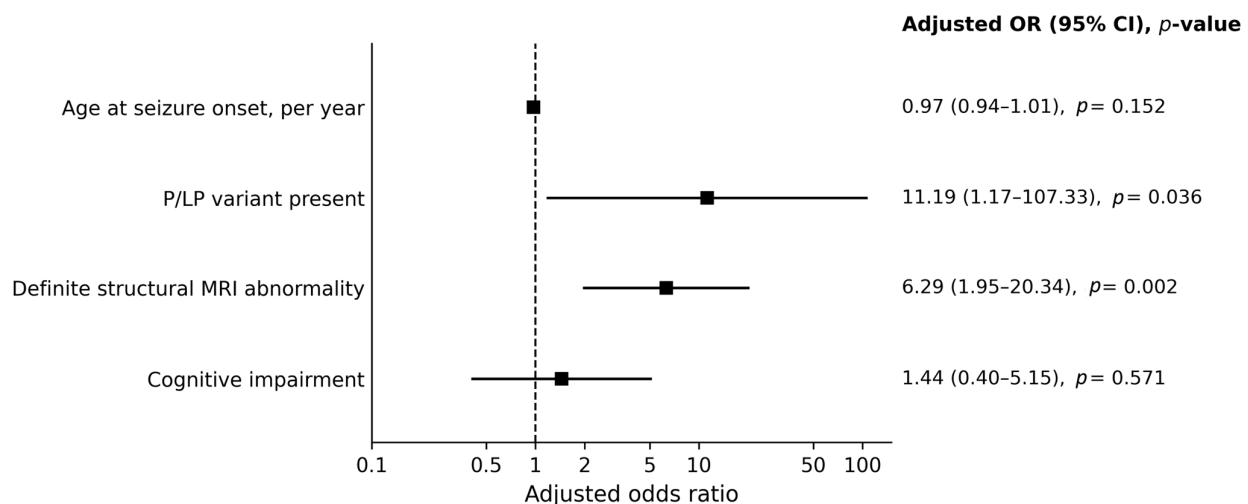


Figure 4. Exploratory multivariable logistic regression model for drug-resistant epilepsy. Forest plot showing adjusted odds ratios and 95% confidence intervals for variables selected a priori: age at seizure onset, presence of a pathogenic or likely pathogenic variant, definite structural MRI abnormality, and cognitive impairment. The x-axis is presented on a logarithmic scale. The vertical dashed line indicates an odds ratio of 1. DRE, drug-resistant epilepsy; P/LP, pathogenic or likely pathogenic; OR, odds ratio; CI, confidence interval.

4. Discussion

In this real-world epilepsy cohort with complex neurological manifestations from Southern Kazakhstan, proband-only WES provided a diagnostically relevant finding in 15.4% of patients when diagnostic yield was defined strictly by the presence of pathogenic or likely pathogenic variants. VUS were identified in an additional 20.5% of patients and were reported separately rather than considered definitive molecular diagnoses. Patients with DRE showed a higher frequency of P/LP variants and definite structural MRI abnormalities than drug-responsive patients. In the exploratory multivariable model, both the presence of P/LP variants and definite structural MRI abnormality were associated with DRE after adjustment for age at seizure onset and cognitive impairment. These findings indicate the potential clinical relevance of WES as part of etiological evaluation in selected patients with epilepsy and complex neurological manifestations, consistent with growing evidence that genetic testing can be informative in clinically selected epilepsy cohorts [27–29]. Most P/LP findings and CNVs identified in this cohort involved genes or genomic regions previously reported in association with epilepsy, neurodevelopmental disorders, or syndromic neurological phenotypes, supporting their interpretation in the context of ACMG/AMP criteria, inheritance data, and genotype–phenotype concordance.

The diagnostic yield of 15.4% in the present cohort falls within the range reported in epilepsy studies, from approximately 10% in broader cohorts to around 30% in selected

exome-based cohorts enriched for presumed genetic etiologies [27–30]. This variability likely reflects differences in cohort selection, phenotype severity, age at seizure onset, neurodevelopmental comorbidities, and testing strategy. The comparatively conservative yield in our cohort may reflect the real-world outpatient recruitment model, phenotypic heterogeneity, and the use of proband-only WES. Importantly, VUS were not included in the diagnostic yield, providing a stricter estimate of clinically relevant molecular findings.

A notable finding of this study was the higher frequency of P/LP variants among patients with DRE compared with drug-responsive patients. This association remained evident in the exploratory adjusted model, in which the presence of P/LP variants was associated with higher odds of DRE after adjustment for age at seizure onset, definite structural MRI abnormality, and cognitive impairment. Definite structural MRI abnormality was also associated with DRE in the adjusted model. These results should not be interpreted as evidence that genetic or structural findings independently predict treatment resistance in all epilepsy populations. Rather, they indicate that, in this cohort, DRE clustered with markers of underlying molecular and structural etiology, consistent with previous evidence linking genetic and structural mechanisms to epileptogenesis and pharmacoresistance [4,11–14].

The therapeutic profile further supports the clinical complexity of the cohort. All patients with DRE were receiving polytherapy, indicating a high treatment burden in this subgroup. However, the study was not designed to compare the efficacy of individual antiseizure medications. Rather, the therapeutic data provide real-world context for interpreting DRE alongside genetic and neuroimaging findings. The predominance of carbamazepine, followed by valproic acid and levetiracetam, reflects routine antiseizure medication use in the regional outpatient setting. In this context, persistent drug resistance may reflect underlying etiological complexity rather than insufficient pharmacological exposure alone.

Earlier age at seizure onset was observed in the DRE group in the unadjusted comparison, but it was not significantly associated with DRE after adjustment for the presence of P/LP variants, definite structural MRI abnormality, and cognitive impairment. This pattern may reflect overlap between early onset and other indicators of etiological complexity, including genetic and structural abnormalities. Cognitive impairment and behavioral/neuropsychiatric features were frequent in the cohort, indicating a high burden of complex neurological phenotypes; however, cognitive impairment was not significantly associated with DRE in the adjusted model. This may be related to the binary classification of cognitive status, overlap with other clinical and molecular variables, or limited sample size.

Structured HPO-based phenotyping was an important component of the diagnostic workflow in this real-world cohort [24]. In epilepsy cohorts, clinical information may be fragmented across historical records, outpatient documentation, EEG reports, and neuroimaging findings, while seizure and comorbidity profiles may be heterogeneous. Manual HPO annotation provided a standardized framework for organizing seizure-related features, neurological findings, cognitive and behavioral manifestations, motor abnormalities, and additional systemic features. In this study, reverse phenotyping was used to assess concordance between candidate molecular findings and the observed clinical phenotype, rather than to reclassify VUS as definitive diagnoses, consistent with cautious variant interpretation under ACMG/AMP principles [25,26].

The real-world outpatient design is a key feature of this study. Unlike highly selected tertiary referral cohorts, the present cohort was identified through routine neurology practice across multiple outpatient centers, which may better reflect the diagnostic challenges encountered in everyday outpatient epilepsy care. Recent registry-based data from Southern Kazakhstan have also highlighted the clinical and psychosocial burden of epilepsy in

this regional context, including the influence of structural healthcare factors on patient outcomes [31]. In this setting, proband-only WES combined with structured phenotyping provided potentially clinically relevant diagnostic information despite the absence of routine trio sequencing and standardized epilepsy-specific MRI protocols. This supports the feasibility of a pragmatic diagnostic workflow for selected patients with epilepsy and complex neurological manifestations in settings where comprehensive genomic resources are not consistently available. In this regional context, access to ISO 15189-accredited clinical molecular genetics laboratories providing advanced genomic testing is limited in routine outpatient practice, and referral to external laboratories may be associated with additional time, cost, and logistical barriers. In this context, WES performed in adulthood may still be clinically informative for patients who remained without etiological diagnosis because earlier genetic testing was unavailable or delayed [32].

The interpretation of VUS remains a major challenge in real-world epilepsy genetics. In this study, VUS were identified in 20.5% of patients but were not included in the diagnostic yield and were not considered definitive molecular diagnoses. This conservative approach is particularly important in proband-only WES, where inheritance information may be incomplete and segregation analysis is not always feasible. Some VUS may become clinically relevant after additional segregation data, functional studies, accumulation of further cases, or future reclassification; however, their current interpretation should remain cautious, consistent with ACMG/AMP principles and prior work emphasizing the value of reinterpretation in epilepsy genetics [25,26,29]. Given the complexity of VUS interpretation, extended bioinformatic reanalysis of uncertain variants is planned as the next stage of this project. This future analysis will include multiple complementary variant-curation platforms, including Franklin/Genoox, manual filtering, updated databases, literature review, segregation data where available, and detailed genotype–phenotype reassessment.

Limitations. This study has several limitations. First, the sample size was modest, and the number of patients with P/LP variants was small, resulting in wide confidence intervals in the logistic regression model. Therefore, the adjusted associations should be interpreted as exploratory rather than definitive predictors of DRE. Second, WES was performed using a proband-only approach; trio-based sequencing was not routinely available, which limited the ability to resolve inheritance patterns and reclassify uncertain variants. Third, EEG and MRI data were obtained from routine clinical practice across multiple outpatient centers, and acquisition protocols were not standardized. Fourth, cognitive impairment was analyzed as a binary variable, which may not capture the full spectrum of neuropsychological function in patients with epilepsy. Fifth, therapeutic data were analyzed descriptively, and the study was not designed or powered to compare the efficacy, tolerability, or pharmacogenomic relevance of individual antiseizure medications. Finally, the cohort was not population-based and may be enriched for patients with clinically complex or treatment-resistant epilepsy who were identified for etiological clarification.

Despite these limitations, this study provides real-world evidence that WES can contribute to etiological evaluation in selected patients with epilepsy and complex neurological manifestations in a resource-limited outpatient setting. The diagnostic contribution was defined conservatively using only P/LP variants, while VUS were reported separately. The association of DRE with the presence of P/LP variants and definite structural MRI abnormality underscores the relevance of integrating clinical phenotyping, neuroimaging, and genomic testing when evaluating patients with epilepsy and complex neurological manifestations or treatment resistance. Rather than defining the genetic architecture of epilepsy in Southern Kazakhstan, these findings support the feasibility and clinical relevance of a structured diagnostic workflow based on phenotyping-guided WES interpretation.

5. Conclusions

In this real-world epilepsy cohort with complex neurological manifestations from Southern Kazakhstan, phenotype-guided proband-only WES provided a diagnostic yield of 15.4% based strictly on pathogenic or likely pathogenic variants. DRE was common and was accompanied by a high burden of polytherapy. P/LP variants and definite structural MRI abnormalities were more frequent among patients with DRE and were associated with DRE in exploratory adjusted analysis. VUS were reported separately and were not considered definitive molecular diagnoses. These findings suggest the potential clinical utility of integrating therapeutic profiling, structured phenotyping, neuroimaging, and WES for etiological evaluation of selected patients with epilepsy and complex neurological manifestations or DRE in resource-limited settings. Larger studies using trio-based sequencing, standardized neuroimaging, pharmacogenomic assessment, and longitudinal follow-up are needed to validate these results.

6. Patents

The findings of the present study enabled the development of a stepwise clinical-genetic diagnostic algorithm for epilepsies with suspected genetic etiology. This algorithm represents an extension and refinement of our previously described diagnostic framework developed for complex neurological phenotypes [33], which was adapted in the present study specifically for the evaluation of epilepsies with suspected genetic etiology. The final version has been registered as intellectual property under Copyright Certificate No. 63721 (3 November 2025), issued by the Ministry of Justice of the Republic of Kazakhstan (Figure S1).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijtm6030030/s1>, Table S1. Clinical characteristics of the study cohort, Table S2. Molecular Findings Identified in the Study Cohort, Figure S1. Stepwise clinical-genetic diagnostic algorithm for epilepsies with suspected genetic etiology.

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Abbreviations

The following abbreviations are used in this manuscript:

ACMG/AMP	American College of Medical Genetics and Genomics/Association for Molecular Pathology
AD	Autosomal dominant
AR	Autosomal recessive
ASM	Antiseizure medication
CMA	Chromosomal microarray analysis
CNV	Copy number variant
DRE	Drug-resistant epilepsy
EEG	Electroencephalography
HPO	Human Phenotype Ontology
ILAE	International League Against Epilepsy
IQR	Interquartile range
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
NEG	Negative WES result
NGS	Next-generation sequencing
OR	Odds ratio
aOR	Adjusted odds ratio
P/LP	Pathogenic or likely pathogenic
SD	Standard deviation
SNV	Single-nucleotide variant
SPSS	Statistical Package for the Social Sciences
VUS	Variant of uncertain significance
WES	Whole-exome sequencing
χ^2	Chi-square test

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