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Diagnosis of Epilepsy in Egyptian Patients: Insights from Whole-Exome Sequencing

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Abstract

Background

Epilepsy is a prevalent and heterogeneous neurological disorder, defined by an enduring predisposition to generate unprovoked epileptic seizures, accompanied by significant neurobiological, cognitive, psychological, and social consequences. It represents the most frequent chronic neurological condition in childhood, affecting approximately 0.5%−1% of children globally. Given its diverse etiologies, molecular diagnosis is crucial for effective management and prognostication.

Objectives

This study aimed to investigate the clinical characteristics and define the underlying genetic etiology of hereditary epilepsy in three unrelated Egyptian families using Whole-Exome Sequencing (WES) as a primary diagnostic tool.

Subjects and Methods

Five patients with epilepsy from three unrelated Egyptian families were enrolled. WES was initially performed on one index patient from each family. Subsequent segregation analysis, utilizing PCR amplification followed by Sanger sequencing, was extended to affected relatives whenever possible.

Results

WES successfully identified three distinct variants across three different genes. A novel likely pathogenic homozygous nonsense variant, c.912G>A; p.(Trp304*) (NM_000218.3) in the KCNQ1 gene was identified in patients 1 and 2 (Family 1). This novel variant was confirmed to be absent from HGMD, ClinVar, and gnomAD databases. Two previously reported variants were also detected: the pathogenic heterozygous missense c.1181C>T; p.(Ala394Val) (NM_001165963.4) in the SCN1A gene in patients 3 and 4 (Family 2), and the likely pathogenic heterozygous missense c.335C>T; p.(Thr112Ile) (NM_003002.4) in the SDHD gene in patient 5 (Family 3). We note that segregation analysis could not be performed for F1and F3 due to the unavailability of parental DNA, which limits the diagnosis confidence in these families

Conclusion

WES proved to be an efficient diagnostic strategy for elucidating the complex, heterogeneous molecular etiology of hereditary epilepsy, particularly in cases lacking a prior genetic diagnosis. Our findings successfully established a molecular diagnosis for the included patients, identified a novel pathogenic variant, and underscored the considerable molecular diversity underlying epilepsy and associated neurological phenotypes within this specific Egyptian cohort.

Keywords: Epilepsy, Whole-Exome Sequencing, WES, neurological disorder, long QT syndrome.

Introduction

Epilepsy is an assortment of prevalent and numerous neurological illnesses marked by daily, unprovoked convulsions [1]. In 2016, epilepsy cases reached around 46 million people globally [2]. In Arabs, the prevalence and incidence of epilepsy were 6.9 per 1000 and 89.5 per 100000, respectively, while the risk factors of epilepsy were positive consanguinity, family history, and history of prenatal injuries and illnesses [3]. The male-to-female ratio globally is nearly one; nevertheless, structural anomalies were more probable in males, especially in the 30- to 59-year-old cohort. [4]. Epilepsy is classified based on onset (unknown, generalized, or focal), type (focal, generalized, or combined focal and generalized), etiology (genetic, structural, infectious, immune, metabolic, or unknown), and specific epilepsy syndromes. This classification system helps guide diagnosis, treatment, and prognosis for epilepsy [5].

The genetic etiology of epilepsy is complex; mutations in numerous genes were revealed to cause epilepsy. These genes are either causing epileptic seizures as the basic manifestation or leading to epilepsy as a part of a general syndrome [1].

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Mutations in genes expressed to ion channels, which maintain neuronal excitability, cause primary epileptic symptoms through dysfunction of these ion channels, causing channelopathies. Gain or loss of voltage-gated sodium, potassium, calcium and chloride ion channels and others leads to various syndromes of epilepsy [6]. Mutations of KCNQ2 and KCNQ3 genes cause benign familial neonatal seizures (BNFS) [7]. Partial seizures take place due to alterations in CHRNA4 or CHRNB2 genes [8]. Mutations of SCN1A and SCN1B genes, which are expressed in sodium channels, also cause channelopathies with variable degrees of epilepsy [9]. Different levels of epilepsy take place by mutations in the KCNB1, KCNT1, CACNA2D2, and HCN1 genes associated with potassium, potassium, calcium, and cation channels, respectively [6]. On the other hand, to a lesser extent, mutations in genes coding non-ion channel proteins lead to epileptic syndromes such as PCDH19, ARX, STXBP1, and X-linked CDKL5 genes [6].

Moreover, mutations in other genes cause epilepsy as a part of a general syndrome, such as *GNAQ, GRIN2A, MECP2, TSC1, SCN1A, GFAP,* and *KCNQ1* genes causing Sturge-Weber Syndrome (OMIM#185300). [10], Landau-Kleffner Syndrome (OMIM#245570) [11], Rett Syndrome (OMIM#312750) [12], Tuberous Sclerosis Complex (OMIM#191100) [13], Dravet Syndrome (OMIM#607208) [14], Alexander Disease (OMIM#203450) [15], and Jervell and Lange-Nielsen Syndrome (OMIM#220400) [16], respectively

Defects of the mitochondrial respiratory chain impair the metabolism of energy, affecting tissues that require an excessive amount of energy, especially the brain, and frequently result in seizures. These disorders are caused by mutations in nuclear- and mitochondrial-encoded genes. Seizures are accompanied by some of these diseases, such as mitochondrial complex 2 deficiency, nuclear type 3 (OMIM#619167) [17].

In this study, whole-exome sequencing (WES) was successfully conducted on the index patients from three unrelated Egyptian families enrolled in this research. Three distinct pathogenic or likely pathogenic variants were identified across three different genes: *KCNQ1, SCN1A,* and *SDHD.* A novel nonsense variant, p.(Trp304*), was detected in the *KCNQ1* gene. Additionally, two previously reported missense variants were identified, p.(Ala394Val) and p.(Thr112Ile) in the *SCN1A* and *SDHD* genes, respectively.

Subjects and Ethical Considerations

This study enrolled five patients from three unrelated families, who were referred to the Neurology Clinic at the National Research Centre (NRC) in Cairo, Egypt. Prior to their participation, all patients underwent a comprehensive clinical examination and a detailed medical history assessment, which included the construction of a three-generation pedigree.

Ethical approval for this research was obtained from the Medical Research Ethics Committee at the National Research Center (approval no. 044101223). All participants provided written informed consent, in strict adherence to the ethical principles outlined in the 1964 Helsinki Declaration and its subsequent amendments, as well as comparable national and institutional ethical standards. Obtaining informed consent for genetic research is a complex process that necessitates clear communication regarding potential risks, the intended use of data and samples, and the handling of incidental findings [18], Ethical Challenges in Obtaining Informed Consent for Genetic/Genomic Research | ELSIhub n.d.).

DNA Isolation and Whole Exome Sequencing

Peripheral blood samples were collected from all participating patients and their available relatives. Total genomic DNA was extracted from these blood samples using a standard, well-established method [19]. The isolated DNA was then utilized for whole-exome sequencing (WES) and subsequent segregation analysis of identified mutations in the proband's available relatives.

Whole exome sequencing was performed on probands' sample using the xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA, USA) for exome capture. Sequencing was carried out on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA), generating 2 x 100 bp paired-end reads with an output of 12 GB per sample and a minimum coverage depth of 20x. Approximately 99.5% of the targeted bases were covered to that depth. Raw fastq reads were aligned to the human reference genome (GRCh37/Hg19) using bcl2fastq [19]. Alignments were converted to BAM format using Picard. VCF files were generated using The Genome Analysis ToolKit (GATK's) HaplotypeCaller (v4.1.8.0) [20]. The American College of Medical Genetics and Genomics guidlines were used for variant pathogenicity classification. All variants were checked in the population databases (1000Genomes, ExAC, ESP6500, GnomAD v.4.1.0) [21]. Variants were reviewed in three databases: ClinVar database (https://www.ncbi.nlm.nih. gov/clinvar/), Franklin by Genoox (https://franklin.genoox. com), and VarSome (https://varsome.com/). WES is a widely recognized and effective diagnostic approach for identifying molecular defects in individuals with suspected genetic disorders [21, 22]

Variant Analysis and Interpretation

All generated sequencing reads were meticulously mapped against the human reference genome (GRCh37/hg19). Variant annotation, a critical step in identifying disease-causing mutations, was performed using ANNOVAR to predict the location and functional impact of each variant [23].

Only variants deemed relevant to the patients' specific clinical phenotypes were selected for further evaluation. Novel variants were systematically queried against multiple established public databases, including ClinVar [24], HGMD [25], gnomAD [26], 1000 Genomes [27], GME Variome [28], and ExAC [29]. Final variant classification was performed according to the 2015

American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines. Variants were classified as Pathogenic, Likely Pathogenic, Variant of Uncertain Significance (VUS), Likely Benign, or Benign. The evidence codes used for classification were recorded and are presented in Table 1.

Confirmation of WES Results via Sanger Sequencing

To confirm the whole exome sequencing findings and perform segregation analysis, Sanger sequencing was conducted on available family members. The detected variants were amplified using polymerase chain reaction (PCR) with specific primers. The primer pair of the SCN1A gene was: SCN1A-12F:5'-ATGAGTAAATTGAAAGTTGAAGCC-3' and SCN1A-12R 5'-TCCTCATACAACCACCTGCTC-3'. PCR products were subsequently visualized on a 2% agarose gel to confirm successful amplification. Amplified products underwent purification using the Exo_SAP purification protocol and were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). The resulting sequences were analyzed and compared against reference sequences using sequence analysis software v5.4 to validate the WES findings and confirm variant segregation within the families.

Results

This study included five Egyptian cases with epilepsy from three unrelated Egyptian families. They revealed a novel likely pathogenic homozygous nonsense c.912G>A; p.Trp304* variant in the *KCNQ1* gene (NM_000218.3; OMIM#607542) in patient 1 and patient 2 from family 1 (F1).This variant was not reported in HGMD, gnomAD (including gnomAD v4.1.0), dbSNP or ClinVar databases. Moreover, two previously reported variants were also found: the pathogenic heterozygous missense c.1181C>T; p.(Ala394Val) variant in the *SCN1A* gene (NM_001165963.4; OMIM#182389) in patient 3 and patient 4 from family 2 (F2) and the likely pathogenic heterozygous missense NM_003002.4:c.335C>T; p.Thr112Ile in the *SDHD* gene (NM_003002.4; OMIM#602690) in patient 5 from family 3 (F3) table (1).

Table 1: Genotype details of the three variants included in this study

Family ID	Gene	Variant (hg38)	Variant Type	Zygosity	Clinical Diagnosis	ACMG classification	ClinVar dbSNP ID	gnomAd (v4.1.0)	Revel
F1	***KCNQ1*** OMIM ID: 607542	chr11-2572977 G>A NM_000218.3:c.912G>A p.Trp304* exon 6/16	Nonsense	AR Homozygous	Jervell and Lange-Nielsen syndrome OMIM ID: 220400	Likely Pathogenic (PVS1, PM2)	Not found	Not found	---
F2	***SCN1A*** OMIM ID: 182389	chr2-166903476 G>A NM_001165963.4: c.1181C>T p.Ala394Val exon 12/29	Missense	AD Heterozygous	Generalized epilepsy with febrile seizures plus, type 2, Febrile seizures, familial, 3A OMIM ID: 604403	Pathogenic (PS4, PM2, PM1, PP3, PP2, PP5)	LP: 2 rs2105862847	Not found	0.87 (Deleterious) (Moderate)
F3	***SDHD*** OMIM ID: 602690	chr11-112094825 C>T NM_003002.4:c.335C>T p.Thr112Ile exon 4/4	Missense	AR Heterozygous	Mitochondrial complex 2 deficiency, nuclear type 3 OMIM ID: 619167	Likely Pathogenic (PM1, PP2, PM2, PP3)	VUS:1 rs199869408	0.00001303	0.87 (Deleterious) (Moderate)

Family 1 (F1), a distant consanguineous family from the same village, includes a living normal older male, 8 years old, and affected female and male children who died at ages 3.5 and 2 years (patients 1 and 2), respectively (Fig. 1). WES analysis was performed for the younger son before his death and revealed a novel likely pathogenic homozygous nonsense c.912G>A; p.Trp304* variant in the KCNQ1 gene (NM_000218.3; OMIM#607542). The c.912G>A variant is associated with an autosomal recessive long QT syndrome.

The clinical presentation of the 2-year-old male infant, including recurrent syncope and generalized tonic-clonic epilepsy, delayed speech, and sensorineural hearing loss (SNHL), along with the family history of a sister's sudden death at age 3.5 with similar symptoms, is highly suggestive of Jervell and Lange-Nielsen syndrome (JLNS) (OMIM#220400). This diagnosis is further supported by the sister's history of congenital SNHL and long QT interval on her electrocardiogram (ECG), which are hallmark features of JLNS [30]. According to genotype phenotype correlations, The c.912G>A variant is regarded as a deleterious mutation including non-functional protein, and causing the most serious symptoms of the Jervell and Lange-Nielsen syndrome (JLNS).

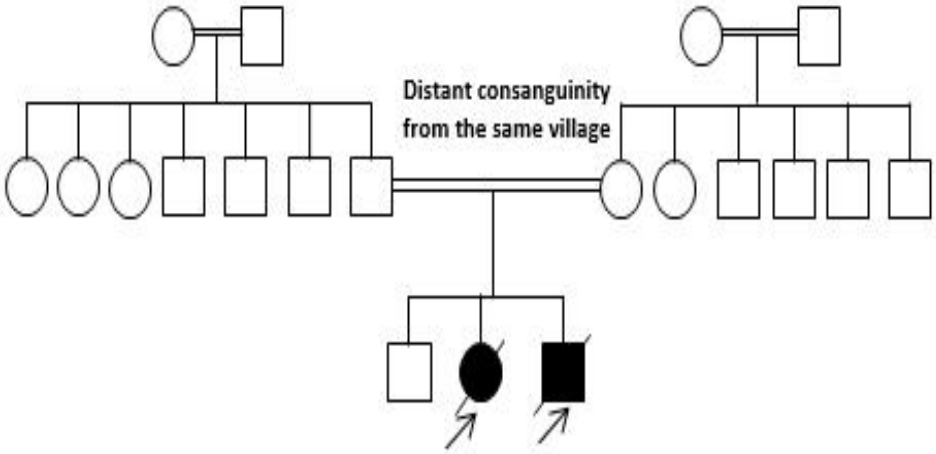

Fig.1 Family#1 Pedigree. The pedigree represented an affected male (black square) and an affected female (black circle). Males are represented by squares, and females are represented by circles. White-coloured squares and circles indicate apparently normal family members, while black colour indicates affected cases. Arrows indicated the probands. A diagonal line (a slash) across a square presented a deceased male and diagonal line (a slash) across a circle presented a deceased female.

Family 2 (F2), a non-consanguineous family, comprises two affected male children aged 15 and 13 (patients 3 and 4), along with their affected mother, who is 43 years old (Fig. 2). Whole exome sequencing analysis (WES) revealed that proband has a very rare previously reported pathogenic heterozygous missense c.1181C>T; p.(Ala394Val) variant in the SCN1A gene (NM_001165963.4; OMIM#182389) (variant details are summarized in Table 1). Segregation indicated an inheritance of the detected mutation from the mother, and its segregation in the proband's brother (Fig. 3). The c.1181C>T variant is associated with an autosomal dominant predisposition to generalized epilepsy with febrile seizures plus, type 2 (OMIM# 604403), with incomplete penetrance and variable severity within affected families. The older son has well-controlled generalized epilepsy. The mother experienced a few febrile seizures during infancy. The younger son had approximately five febrile seizures in infancy and had a single generalized tonic-clonic seizure lasting 10-20 seconds about two years ago. Mother's father had seizures as an infant and began experiencing seizures again in his 20s. His epilepsy is well managed with anticonvulsants. He had seven siblings; one sister and two brothers also have epilepsy. One of his brothers with epilepsy also had diabetes and died from heart disease.

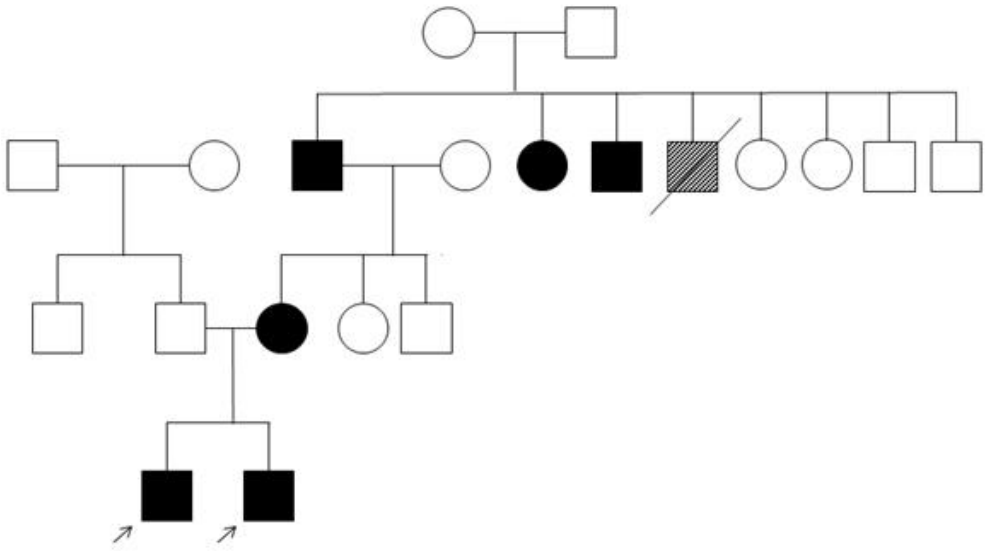

Fig. 2 Family#2 Pedigree. The pedigree represented an affected male (black square) and an affected female (black circle). Males are represented by squares, and females are represented by circles. White-coloured squares and circles indicate apparently normal family members, while black colour indicates affected cases. Arrows indicated the probands. A diagonal line (a slash) across a square presented a deceased male.

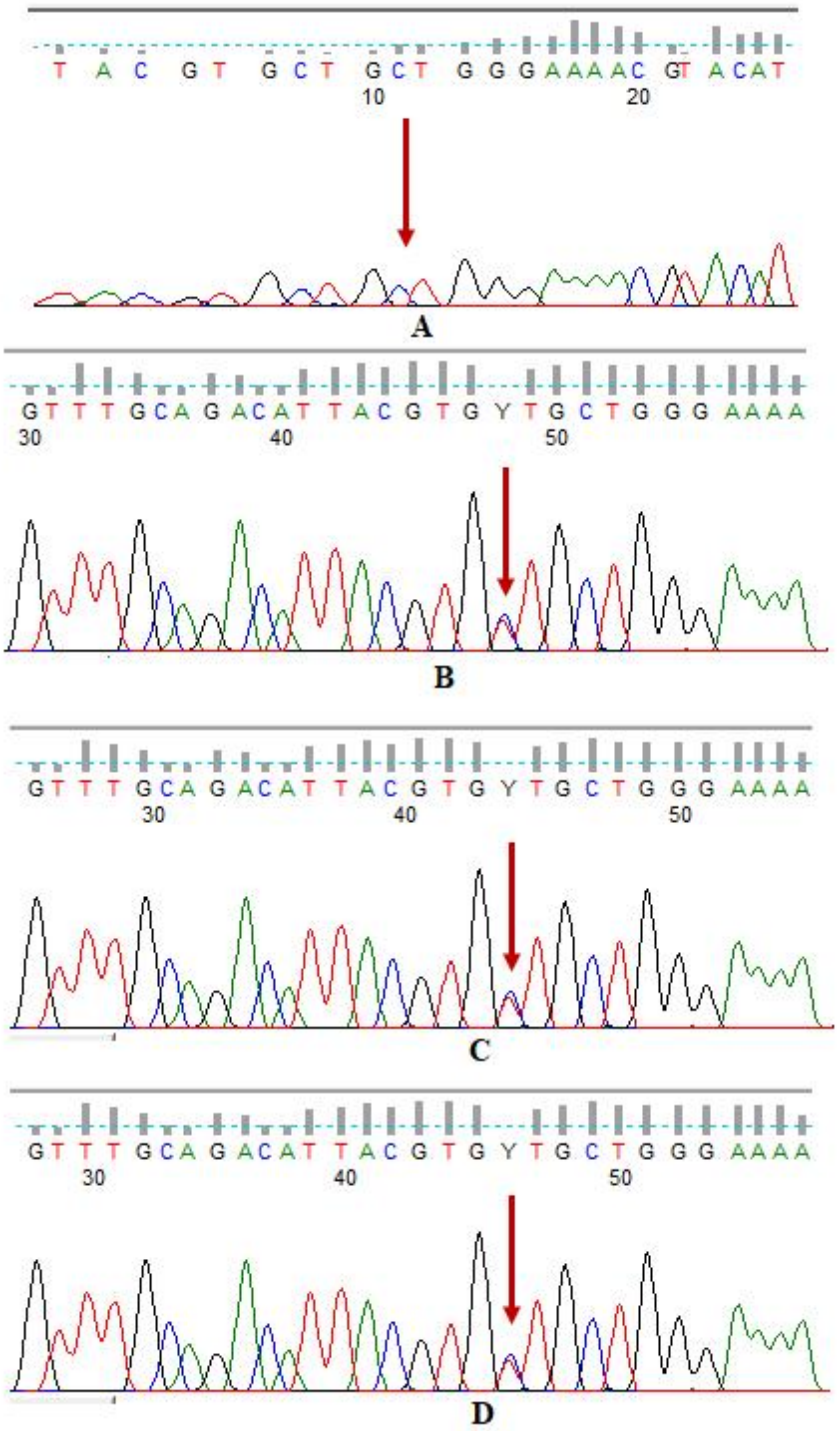

Fig. 3 Sequencing electropherograms showing NM_001165963.4 (SCN1A): c.1181C>T (p.Ala394Val) variant in the SCN1A gene in family 2. (A): Homozygous wild allele in maternal grandmother; (B) Heterozygous allele in the affected mother; (C) Heterozygous allele in the affected older son (Patient 3), and (D) Heterozygous allele in the affected younger son (Patient 4). The arrow indicates the site of base substitution.

The index patient from Family 3 (F3), a 10-year-old male (Patient 5) born to non-consanguineous parents, presented with a complex and severe neurodevelopmental and epileptic phenotype (Fig. 4). The patient's clinical manifestations were characterized by developmental and epileptic encephalopathy, including verbal apraxia, focal and slurred speech, a pronounced nasal tone, and cheilopathy with dysarthria, indicating significant bulbar involvement. Neurological examination further revealed dystonia, incoordination, right eye rolling, and ptosis. The patient also exhibited a generalized epileptic discharge on electroencephalogram (EEG) and significant intellectual disability. An important preceding factor noted in the

history was the presence of a sleep disturbance prior to the onset of the primary neurological symptoms. WES identified a previously reported likely pathogenic heterozygous missense variant, NM_003002.4:c.335C>T; p.(Thr112Ile), in the SDHD gene (NM_003002.4; OMIM#602690). Based on the comprehensive clinical profile and the identified genetic variant, the patient's presentation is consistent with a diagnosis of Mitochondrial Complex II Deficiency, Nuclear Type 3 (OMIM#619167).

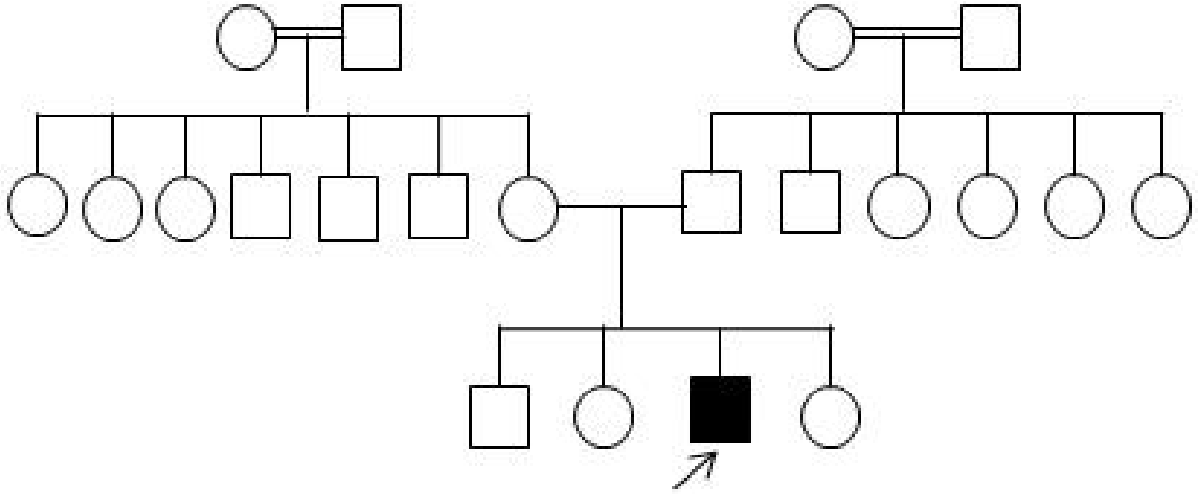

Fig. 4 Family#3 Pedigree. The pedigree represented an affected male (black square) and an affected female (black circle). Males are represented by squares, and females are represented by circles. White-coloured squares and circles indicate apparently normal family members, while black colour indicates affected cases. Arrow indicated the proband.

Discussion

Epilepsy is a widespread brain disorder, with at least two spontaneous seizure and a high likelihood of repeated seizures. It impacts people at any age [31]. Epileptic attacks are brought on by a variety of disorders and traumas. In 50% of cases, the driver of the illness is unexplained [2]. The genetic etiology of epilepsy is expanding to include mutations in genes that cause epilepsy as the main symptom or alterations in genes that result in epilepsy as a component of another syndrome [1].

In the current study, five Egyptian patients with epilepsy from three unrelated Egyptian families revealed three disease-causing variants. Patients 1 and 2 (F1) had a novel, likely pathogenic homozygous nonsense c.912G>A; p.Trp304* variant in the KCNQ1 gene (NM_000218.3; OMIM#607542), which results in a premature stop codon (p.Trp304*). Patients 3 and 4 (F2) had the pathogenic heterozygous missense c.1181C>T; p.(Ala394Val) variant in the SCN1A gene (NM_001165963.4; OMIM#182389), while patient 5 (F3) revealed the likely pathogenic heterozygous missense c.335C>T; p.Thr112Ile in the SDHD gene (NM_003002.4; OMIM#602690) (Table 1). The KCNQ1 gene encodes a 676-amino acid protein called Potassium voltage-gated channel subfamily KQT member 1 (UniProtKB/Swiss-Port: P51787), which regulates the excitability of the cardiomyocyte and is vital for proper development and performance of the heart, colon, inner ear, and stomach [32, 33].

JLNS (OMIM # 220400) [30] is a possibly fatal syndrome distinguished by congenital hearing loss and a long QT interval on electrocardiograms (ECGs). Stress, whether bodily or mental, can result in fatal arrhythmia. Biallelic alterations in the KCNQ1 or KCNE1 genes are the trigger of JLNS. In spite of beta-blocker treatment, over 25% of JLNS instances encounter a sudden demise. Cardiac episodes start at a very young age [30]. These manifestations agree exactly with the clinical symptoms of patients 1 and 2 (F1) having sensorineural hearing loss and a long QT interval on ECG. The early deaths of the siblings at ages 3.5 and 2 years are tragically consistent with the life-threatening nature of untreated JLNS. On the other hand, Patients 1 and 2 were diagnosed with recurrent episodes and epilepsy, which agrees with Goyal et al. [2012] [34], who revealed a JLNS patient with intractable epilepsy. They showed that case seizures were accompanied by hypotension and pulselessness, which proves the cardiac base of symptoms. Moreover, β-blocker medication ended seizures. Al-Momani et al. [2025] [35] reported a case with JLNS showing epilepsy; however, neurological examinations, including brain MRI and EEG, showed negative results. The family's history of multiple affected children (a deceased son and daughter) and the presence of a living, presumably asymptomatic, older son suggest an autosomal recessive inheritance pattern. This means that both parents, although asymptomatic, are heterozygous carriers of the pathogenic KCNQ1 variant. Affected children inherit one mutated copy from each parent.Several mutations in KCNQ1 gene were reported in JLNS cases from Morocco [36], Turkey [37], Algeria [38], Saudi Arabia [39], and Iran [40].

he SCN1A gene is transcribed to a 2009-amino-acid protein termed sodium channel protein type 1 subunit alpha (UniProtKB/Swiss-Port: P35498). It's vital for proper neuron activity since it governs sodium ion influx, which creates and transmits action potentials [41]. Generalized epilepsy with febrile seizures plus, type 2 (GEFSP2), is caused by monoallelic alteration in the SCN1A gene. It is a neurologic disease distinguished by infantile episodes of seizures with fever. Late in life,

the cases suffer from generalized tonic-clonic seizures with or without fever. There is a variety of seizure conditions ranging from mild to severe according to the type of mutations. Missense ones cause milder episodes, while truncation mutations lead to severe epileptic syndrome [42, 43]. In patients 3 and 4 (F2), there is a very rare heterozygous missense c.1181C>T; p.(Ala394Val) variant in the SCN1A gene (NM_001165963.4; OMIM#182389). This mutation was not published; the cytosine is mutated to thymine, causing conversion of alanine amino acid number 394 to valine. However, Myers et al. [2017] reported another heterozygous missense mutation, in a 13-year-old female case with repeated febrile seizures, in the same position of the SCN1A gene, c.1181C>A; p.(Ala394Asp), in which the cytosine mutated to adenine, converting alanine amino acid number 394 to aspartate [44]. Myers et al. [2017]'s case had mild learning difficulties, mild prominence of lateral brain ventricles, and repeated febrile seizures with absence [44].

Her maternal uncle had also had febrile seizures. In our study, patients 3 and 4 (F2), aged 13 and 15 years, respectively, and their 34-year-old mother and mother's father are also affected, which confirms the autosomal dominant nature of the disorder. Well-controlled generalized epilepsy was reported in the older case, with a few febrile convulsions during infancy. In his mother, while the younger son experienced five febrile seizures in infancy and a single tonic-clonic seizure lasting 10-20 seconds two years ago. Mutation in SCN1A gene causing epilepsy were reported in Tunisia [45], and Saudia Arabia [46].

The SDHD gene is transcribed to a 159-amino acid protein called succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial. It transfers electrons from succinate to ubiquinone (coenzyme Q) in complex II of the mitochondrial respiratory chain [47]. Defects in mitochondrial activities compromise energy production, affecting brain demands of energy, which precipitates seizures. Energy deficiency causes of seizures include oxidative stress, shortage of β-oxidation, glial cell malfunction, neurotransmitter dysfunction, improper calcium signaling, immune-mediated destruction, structural defects in the brain, and variations in cerebral blood circulation [48]. Epilepsy is mediated by alterations of genes encoding the mitochondrial electron transport chain [17]. Mitochondrial complex 2 deficiency, nuclear type 3 (OMIM#619167), is caused by autosomal recessive mutations in the SDHD gene. According to Jackson et al. 2014, it shows a heterogeneous phenotype including encephalomyopathy with seizures or myoclonus, hypotonia, ataxia, dystonia, and loss of motor abilities [49], which agrees with the manifestation of patient 5 (F3) in our study, who has verbal apraxia, focal and slurred speech, a generalized epileptic discharge, a nasal tone, cheilopathy, dysarthria, right eye rolling, ptosis, incoordination, dystonia, intellectual disability, and bulbar involvement. Patient 5 has a likely pathogenic heterozygous missense c.335C>T; p.Thr112Ile in the SDHD gene (NM_003002.4; OMIM#602690). This mutation was proved to be pathogenic by Panizza et al. [2012], who used a yeast model to elucidate the pathogenicity of several mutations in the SDHB, SDHC, and SDHD genes. They reported that the heterozygous missense c.335C>T variant in the SDHD gene causes pheochromocytomas and paragangliomas [50]. The presence of merely a monoallelic mutation in the SDHD gene in patient 5 of our study with GEFSP2 can be explained by deep intronic mutation or copy number variation (CNV), which cannot appear in WES [51]. We acknowledge that in two families (F1 and F3), segregation analysis could not be completed due to the unavailability of DNA from key relatives. In these cases, while the identified variants meet several criteria for pathogenicity, the lack of segregation data reduces the overall diagnostic confidence. Biallelic SDHD variants were revealed in a Palestinian family causing of mitochondrial complex II deficiency [52].

Conclusion

Whole-Exome Sequencing (WES) is an efficient diagnostic strategy for elucidating the complex, heterogeneous molecular etiology of hereditary epilepsy, particularly where the causative gene was initially unknown. In this study, WES successfully identified three distinct variants across three different genes: *KCNQ1, SCN1A,* and *SDHD.* The most significant finding involved the *KCNQ1* gene, where a novel, likely pathogenic homozygous nonsense variant, p.(Trp304*) was identified in one family. Additionally, two previously reported missense variants were detected p.(Ala394Val) in the *SCN1A* gene and p.(Thr112Ile) in the *SDHD* gene. These findings effectively establish a molecular diagnosis for the included patients and underscore the considerable molecular diversity underlying epilepsy and associated neurological phenotypes within this specific Egyptian cohort.

Limitation of this study

This study has a few limitations that should be considered when interpreting the results:

The pathogenic variant p.(Ala394Val) identified in the third family is heterozygous, yet the associated disease is autosomal recessive. This suggests that a second pathogenic variant is likely present but was not detected by Whole Exome Sequencing (WES). This missing variant could reside in deep intronic regions, which are not covered by WES, or it may be due to a large deletion or duplication that isidentified by Copy Number Variation (CNV), which missed by standard WES analysis.

Confirming WES results by Sanger sequencing could not be performed for the first and third families due to sample unavailability. For the first family, the proband (the affected individual) had passed away, making confirmation impossible. For the third family, the necessary samples were also unavailable.

Authors' contributions

This work was conducted collaboratively by all authors. A.K. and E.E.A.M. were responsible for designing the study, developing the protocol, and coordinating the research. A.K., M.A., A.M.A.E. and S.Y.M. carried out the clinical study involving patients and their families and collected the samples. E.E.A.M., N.N.A. and T.H.A.A, performed the molecular

genetics experiments, analyzed the data, interpreted the findings, and searched the literature. T.H.A.A, drafted the initial manuscript, which E.E.A.M. then revised. All authors reviewed, amended, and approved the final manuscript.

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Availability of data and materials

Data is provided within the manuscript or supplementary information files.

Declarations

Human Ethics and Consent to Participate declarations

Written informed consent was obtained from the parents of all participating patients, according to the guidelines of The Medical Research Ethics Committee at the NRC.

Consent for publication

Participants or legal guardians have consented for the publication.

Competing interests

The authors declared that no conflict of interest exists.

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