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Calpain 7 as a novel candidate gene in genetic generalized epilepsy

Faezeh Jamali [1, 2], Mohammad Shariati [3, 4], Mohammadtagi Farzadfard [3], Juliane Winkelmann [5, 6, 7], Mohsen Foroughipour [3], Mir Salar Kahaei [1], Ariane Sadr-Nabavi [1, 2, 3*], Michael Zech [5, 6, 8*]

[1] Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Neurology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Academic Center for Education, Culture, and Research (ACECR)-Khorasan Razavi, Mashhad, Iran
[5] Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany
[6] Institut Für Humangenetik, Technische Universität München, School of Medicine and Health, Munich, Germany
[7] Munich Cluster for Systems Neurology, SyNergy, Munich, Germany
[8] Institute for Advanced Study, Technical University of Munich, Garching, Germany

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ABSTRACT

Objective(s): Genetic generalized epilepsy (GGE) is a common subtype of epilepsy characterized by generalized seizure types, with an unclear etiology and recognized genetic contribution to its susceptibility. Although genetic factors play a significant role, the precise mechanisms and causative variants underlying GGE remain poorly understood. This study aimed to identify the genetic basis of GGE.

Materials and Methods: Whole exome sequencing (WES) was performed in eight consanguineous GGE families. Sanger sequencing was conducted to validate the WES findings and confirm variant segregation within the families. RNA-seq data (GSE185632) and *in silico* analyses were used to assess gene expression and variant pathogenicity.

Results: A rare nonsense variant in exon 13 of Calpain 7 (CAPN7, NM_014296.3: c.1454G>A; p.Trp485Ter) was identified and determined to be pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria (PVS1, PM2, PP4, and PP1). The variant co-segregated with the disease in the family. RNA-seq analysis of epilepsy transcriptomes revealed significant down-regulation of this gene (log2FC= -0.84, padj = 0.016). Complementary computational analyses demonstrated strong evolutionary constraint and pathogenic signatures, further supporting its disease association. CAPN7 negatively perturbate endometrial stromal cell decidualization in epithelial–mesenchymal transition via AKT pathway. The proteolytic activity of CAPN7 is associated with degradation of EGFR.

Conclusion: This study provides novel insight on the association of *CAPN7* in GGE, highlighting its potential contribution to epilepsy pathogenesis. Further research is required to gather additional evidence and elucidate the molecular mechanisms underlying the clinical manifestations associated with *CAPN7* variants.

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Introduction

Genetic generalized epilepsies (GGEs) constitute a common subgroup of epilepsies, accounting for approximately 15%-20% of all cases. GGEs are defined by the presence of generalized seizure forms, such as absence, myoclonic, and generalized tonic-clonic seizures, typically occurring in individuals with unimpaired cognitive function and unremarkable neuroimaging findings (1-3). GGEs refer to a collection of self-limiting epileptic syndromes, such as juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), epilepsy with generalized tonic-clonic seizures (GTCs) alone, and epilepsy with eyelid myoclonia (4, 5). Each syndrome is defined by specific features, including age at onset, characteristic seizure types, and distinctive ictal and interictal electroencephalography (EEG) findings (6-8).

Over the years, extensive research has improved the understanding of the genetic basis of epilepsy, leading to the identification of three major categories of genetic alterations contributing to the disease: Monogenic variants, copy number variants, and common variants. Monogenic variants are estimated to account for approximately 2%-8% of GGEs

*__Corresponding authors:__ Ariane Sadr-Nabavi. Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Department of Neurology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Email: asadrnabavi@gmail.com; Michael Zech. Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany, Institut Für Humangenetik, Technische Universität München, School of Medicine and Health, Munich, Germany. Institute for Advanced Study, Technical University of Munich, Garching, Germany. Email: michael.zech@helmholtz-muenchen.de

(9, 10). Whole-exome sequencing (WES), combined with candidate gene approaches, has identified numerous GGE-associated genes, including *GABRA1*, *GABRG2*, *GABRB3*, *SLC2A*, *SLC12A5*, *EFHC1*, *CACNA1H*, *CACNB4*, *THBS1*, *KCNMA1*, *CHD4*, *GABRD*, *HCN2*, *KCC2*, *CLCN2*, and *RYR2*, which play roles in GABAergic signaling, ion channel regulation, and other essential neuronal processes (11-20).

Despite these advances, the genetic pathophysiology of GGEs remains complex and incompletely understood, with many affected individuals likely harboring pathogenic variants in yet-undiscovered disease-associated genes. This study aimed to identify genetic variants associated with GGEs by performing WES in families with multiple affected individuals.

Materials and Methods

Patients

We recruited eight consanguineous families with GGE, each having at least two affected individuals, for genetic analysis. The diagnosis of GGE was confirmed by two neurologists based on clinical presentation, age at seizure onset, seizure classification, electroencephalography (EEG) characteristics, and neuroimaging results. Peripheral blood samples (5 ml) were obtained from participants following written informed consent, along with detailed clinical data including medical histories and seizure records. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1402.124) and conducted at the neurology and neurogenetics clinics of Ghaem Hospital.

Whole-exome sequencing

Genomic DNA was extracted from blood samples collected from all family members using the Qiagen Flexi Gene DNA kit (Qiagen, Hilden, Germany). Whole-exome sequencing (WES) was conducted for one affected individual from each family. Exome library preparation was carried out using Illumina 100-bp paired-end protocols. The enrichment of exonic and adjacent intronic regions was carried out with the Agilent SureSelect Human All Exon v6 kit (Agilent Technologies, Santa Clara, CA, USA), and sequencing was performed on an Illumina HiSeq 4000 platform at the Helmholtz Center, Munich, Germany.

Data analyses

Data processing was carried out using the established analysis pipelines of the Helmholtz Center Munich and the Technical University of Munich. Using the Burrows-Wheeler Aligner (BWA-MEM), sequencing reads were aligned against the GRCh37 build (hg19) of the reference genome. SAMtools, PINDEL, and the Genome Analysis Toolkit (GATK) were used for calling insertions-deletions (indels) and single nucleotide variants (SNVs), and ExomeDepth was used for detecting copy number variations (CNVs). Genomic variants were annotated considering their chromosomal coordinates, possible functional consequences, and population allele frequencies derived from gnomAD and a local exome database (21).

Following annotation, a bioinformatics filtering pipeline was applied to narrow down the list of variants. Gene lists of interest were compiled through literature review and OMIM database searches, based on relevant phenotypic features. Within these predefined gene sets, variants predicted to alter protein function were retained if their minor allele frequency (MAF) was ≤0.001 for autosomal dominant and X-linked genes, and ≤0.005 for autosomal recessive genes. Computational prediction tools, including CADD, PolyPhen-2, and SIFT, were utilized to further evaluate the variant's pathogenicity. Variants previously reported in databases such as ClinVar and HGMD, or cited in the literature, were also considered. Candidate genes were further prioritized based on gene expression profiles and protein data from the Human Protein Atlas, UniProt, GTEx, and population constraint metrics available in gnomAD (pLI and missense Z-scores)(21).

After completing the final filtering step, exomes were first screened against a curated list of epilepsy-related genes, which yielded no candidate variants. The data were then re-analyzed using broader criteria, including a high CADD score (≥20), predicted loss-of-function variants (e.g., stop-gain and splice-site alterations), and high penetrance assumptions, allowing for all modes of inheritance and considering homozygous variants in accordance with the observed pedigrees. Frequency filtering was applied using a 0.5% threshold based on gnomAD and in-house databases, including the Iranome database. The pathogenic potential of the final candidate variant was evaluated according to the criteria defined by the ACMG, incorporating both institutional classifications and previously published evidence (22).

Differential expression analysis of epilepsy RNA-seq data

Gene expression data (mRNA, lncRNA, and circRNA) from brain tissues of healthy controls and epilepsy patients were retrieved from the GEO database (accession number GSE185632). The dataset included read counts for each gene across all samples. To exclude genes with very low expression levels that could bias the differential expression analysis, a filtering criterion was applied: each gene was required to have a read count of at least 10 in a minimum number of samples. Genes not meeting this criterion were removed from further analysis. Following this, differential expression analysis was carried out with the DESeq2 package within the R environment. The DESeq function was utilized to normalize the data and conduct statistical testing based on the Wald test, applying the sfType="poscount" parameter for library size normalization. To identify statistically significant differentially expressed genes, padj < 0.05 was set as the cutoff, and genes meeting this threshold were deemed differentially expressed (23).

Computational protein structure and variant analysis

The 3D conformations of both the wild-type and mutant proteins were modeled utilizing I-TASSER, which combines multiple threading alignments and structural assembly simulations for structure and function prediction (24). For molecular visualization and structural analysis, the PyMOL Molecular Graphics System was employed (25). Structural comparisons between wild-type and mutant proteins were conducted to identify domain disruptions and conformational changes caused by the variant.

To comprehensively evaluate the potential effects on function of the identified variant, three complementary *in silico* tools were applied: (1) ConSurf, which examines the conservation status of amino acids through comparative sequence alignments of homologous proteins (26); (2)

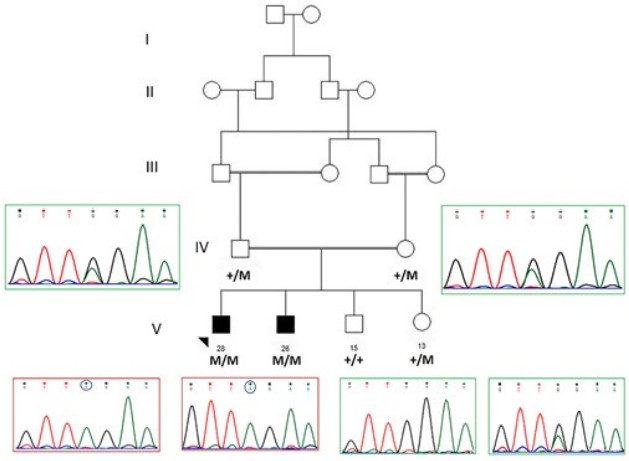

Figure 1. EP-03 Family pedigree and co-segregation results for the CAPN7 variant c.1454G>A (p.Trp485Ter)

MetaDome (v1.0.1), a tool that evaluates domain-specific tolerance to variation using an intolerance threshold of 0.5; and (3) PMut, a neural network–based algorithm developed to assess the structural and functional alterations caused by amino acid substitutions (27, 28).

Results

CAPN7 and genetic

Analysis of exome sequencing and pedigree data from Family EP-03 revealed a homozygous nonsense variant (NM_014296.3: c.1454G>A: p.Trp485Ter) in exon 13 of the calpain 7 (*CAPN7*, MIM: 606400) gene, which was determined to be pathogenic according to the ACMG criteria (PVS1, PM2, PP4 and PP1).

The identified variant was validated by Sanger sequencing and showed complete co-segregation with the disease phenotype in the family. Affected individuals (V-1, V-2) were homozygous for the variant, while both parents (IV-1, IV-2) and individual V-4 were heterozygous. Individual V-3 was homozygous wild-type (Figure 1). The *CAPN7* c.1454G>A variant is absent from both gnomAD and Iranome population databases. These findings suggest an association between *CAPN7* variants and autosomal recessive GGE, supported by segregation analysis and clinical phenotyping.

Clinical characterization of family EP-03

The proband (Patient 1) was a 28-year-old man and the first child of consanguineous parents. He was born by normal vaginal delivery at full term after an uneventful pregnancy. His psychomotor development was normal, but he began experiencing generalized tonic-clonic and myoclonic seizures at the age of two. His EEG showed generalized epileptiform discharges. The patient's seizures have been resistant to medication. At the age of 26, he developed muscle weakness, and subsequent electrodiagnostic studies and muscle enzyme assessments confirmed a diagnosis of myopathy. On current examination, he had severe proximal muscle weakness and was unable to stand unaided. His head circumference was slightly below the normal range, and he had mild intellectual disability. Additionally, he demonstrated a postural tremor, suspected to be drug-induced.

Patient 2, the proband's brother, was a 26-year-old man who exhibited generalized tonic-clonic seizures and myoclonic seizures at two years of age, similar to his brother. The EEG was poly-spike and showed spike and wave abnormalities. His brain MRI was normal. Unlike his brother, the seizures were controlled using Valproic acid. Up to the most recent clinical assessment, no additional manifestations had been observed. Table 1 summarizes the clinical data for the patients in Family EP-03.

Table 1. Clinical data of Family EP-03

	Patient 1	Patient 2
Demographics and family information		
Age	28	26
Gender	Male	Male
Geographical origin (ethnicity)	Iran	Iran
Consanguinity	Yes	Yes
Epilepsy characteristics		
Age of seizure onset	2 y/o	2 y/o
Intractable epilepsy	Yes	No
Seizure burden	Monthly	Yearly
Seizure Type	Generalized Tonic-clonic myoclonic	Generalized Tonic-clonic myoclonic
Seizure duration	6 min	2 min
Brain MRI	Normal	Normal
EEG finding	Generalized spike-wave discharges	Generalized spike-wave discharges
Antiepileptic drug (AED)		
Age starting AED	2 y/o	2 y/o
Current AED	Levetiracetam Phenobarbital, Lamotrigine, Clobazam	Valproic acid
Past AED	Valproic acid, Oxcarbazepine	Phenobarbital, Lamotrigine Oxcarbazepine
Effective AEDs	-	Valproic acid
AEDs: severe adverse effect	No	No
Seizure outcome	Not controlled	Controlled with AED

Differential expression analysis in epilepsy transcriptomes

Differential expression analyses revealed that *CAPN7* (ENSG00000131375) showed a significant decrease in expression in epilepsy patients compared to the control group (log2FoldChange = - 0.84, padj = 0.016; Table 2).

Integrated computational analysis of variant effects

The identified variant (*CAPN7*: c.1454G>A, p.Trp485Ter) was classified as pathogenic based on concordant predictions from multiple *in silico* tools (BayesDel_addAF, BayesDel_noAF, Eigen, Eigen_PC, and FATHMM_MKL databases). Furthermore, its CADD 1.7 score of 45 places it firmly within the pathogenic range, underscoring the potential

Table 2. DESeq2 differential expression output for CAPN7: Epilepsy patients vs healthy controls

GeneID	padj	*P*-value	lfcSE	stat	log2FoldChange	baseMean	Symbol
ENSG00000131375	0.016098	0.001866	0.270547	-3.11083	-0.8416256	946.8163	CAPN7

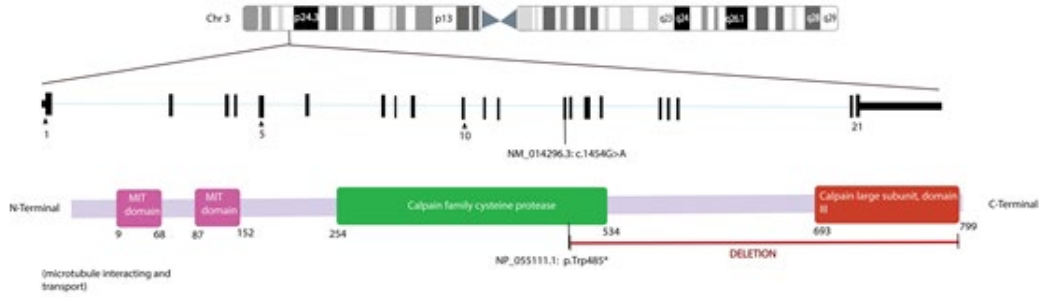

Figure 2. Genomic and protein features of CAPN7
CAPN7 consists of two MIT domains, a catalytic cysteine protease domain, and the calpain large subunit domain III

clinical significance of this genetic alteration. The primary references for these assessments include dbNSFP v4.3 and the UCSC Genome Browser, which collectively integrate multiple lines of evidence to evaluate variant pathogenicity.

This variant is located on chromosome 3 at 3p25.1, within exon 13 of the gene, resulting in a premature stop codon at protein position 485. Consequently, this truncation affects part of the calpain family cysteine protease domain, which is critical for substrate cleavage, as well as the entire calpain large subunit domain III. Both domains are essential for the protein's enzymatic function, structural stability, and substrate interactions (Figure 2).

According to the ConSurf database, the tryptophan residue at the affected site exhibits high conservation across different species, and the additional deleted amino acids in this region likewise show strong evolutionary conservation. These findings support the conclusion that substitutions or deletions in this region are likely to disrupt normal protein function (Figure 3).

Moreover, data from the MetaDome and PMut databases indicate that multiple residues in the deleted region are intolerant to variation and potentially pathogenic, further emphasizing the functional importance of this segment (Figure 4).

Comparative modeling of wild-type and mutant proteins revealed a significant loss of functional domains. The resulting structural alteration is evident and underscores the anticipated loss of normal protein function (Figure 5).

Discussion

To the best of our knowledge this is first study showing the association of *CAPN7* as a new candidate gene for GGE. Through WES, we identified a novel homozygous nonsense variant (c.1454G>A: p.Trp485Ter) in *CAPN7*, exhibiting an autosomal recessive inheritance pattern. Supporting this connection, analysis of the GEO dataset (GSE185632) revealed a significant decrease in *CAPN7* expression in epileptic patients (log2FoldChange=-0.84, padj=0.016), suggesting its potential involvement in epilepsy pathophysiology. Previous studies have underscored the importance of calcium in neuronal excitability and the development of epileptic seizures. Therefore, altered *CAPN7* expression or structural/functional mutations in this gene could affect neuronal mechanisms associated with seizure generation.

CAPN7 belongs to the calpain family, which comprises a set of intracellular calcium-dependent cysteine proteases. The calpains (calcium-dependent proteases with papain-like activity) perform proteolytic cleavage on a variety of

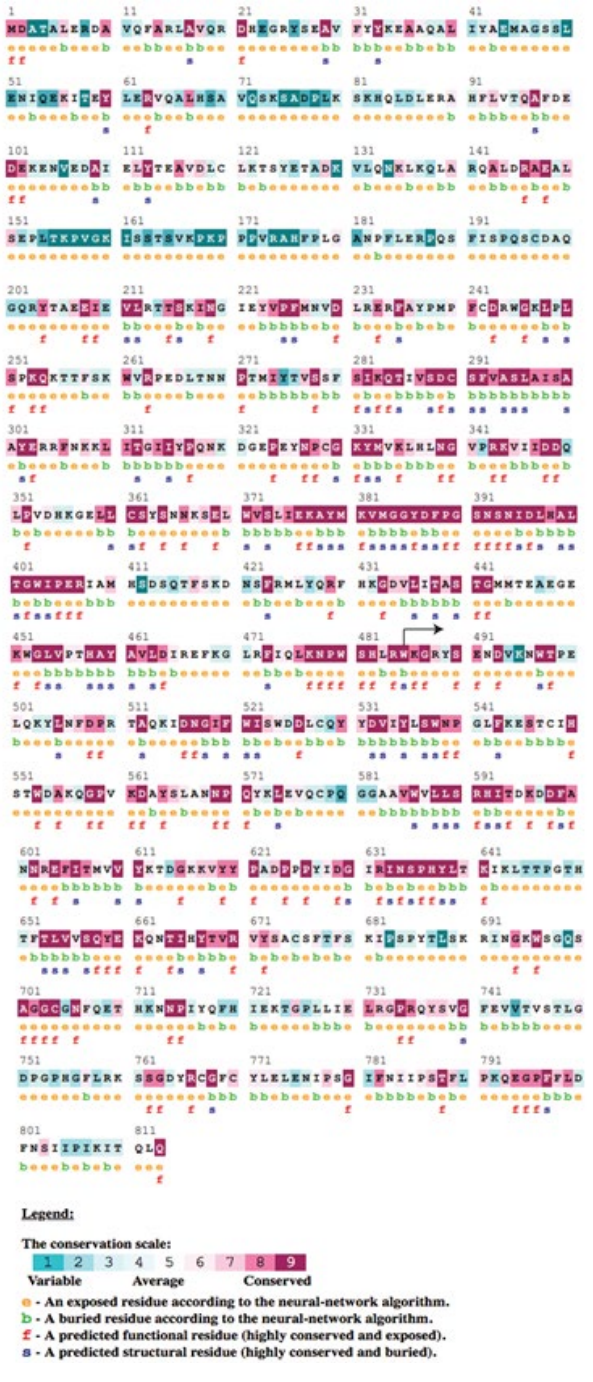

Figure 3. Sequencing conservation analysis of CAPN7 using ConSurf
The color gradient represents conservation scores ranging from 1 (most variable) to 9 (most conserved). The arrow indicates the deleted region

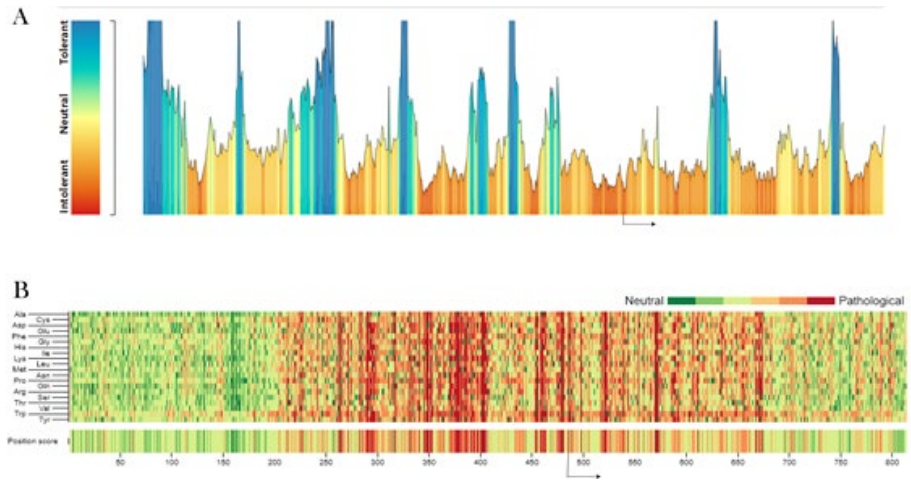

Figure 4. CAPN7 variant tolerance profile from MetaDome. (B) Pathogenicity predictions by PMut. The arrow indicates the deleted region

cellular targets and contribute a crucial role in physiological processes such as signal transduction, the cell cycle, apoptosis, and membrane trafficking (29, 30). The human genome expresses 15 calpain genes, *CAPN1* to *CAPN16* (31). They are divided into two groups based on tissue distribution (tissue-specific and ubiquitous calpains) and domain structures (classical and nonclassical calpains) (32). Mutations in individual calpain genes are associated with calpainopathies that include muscular dystrophies, impaired neurogenesis, lissencephaly, embryonic lethality, gastropathy, tumorigenesis, and deficient sex determination (33).

CAPN7 encodes a protein of 813 amino acid residues and contains 21 exons on chromosome 3p25.1. CAPN7 protein contains two microtubule-interacting and transport domains (MIT), a catalytic cysteine protease domain, and calpain large subunit domain III at its C-terminus (Figure 2). Notably, CAPN7 is a ubiquitous calpain that is predominantly expressed in the pancreas, with moderate expression in skeletal muscle and liver. The function of CAPN7 in humans is still unclear; however, the mouse ortholog is believed to act as a calcium-independent protease. It appears to localize to the nucleus and represents the most divergent member of the calpain superfamily, exhibiting merely 26-35% sequence identity compared to other family members (34). Recent studies, however, have suggested potential roles for CAPN7 in multiple cellular processes. It has been implicated in the degradation of epidermal growth factor receptor (EGFR) and in the negative regulation of endometrial stromal cell decidualization via the AKT signaling pathway (35, 36).

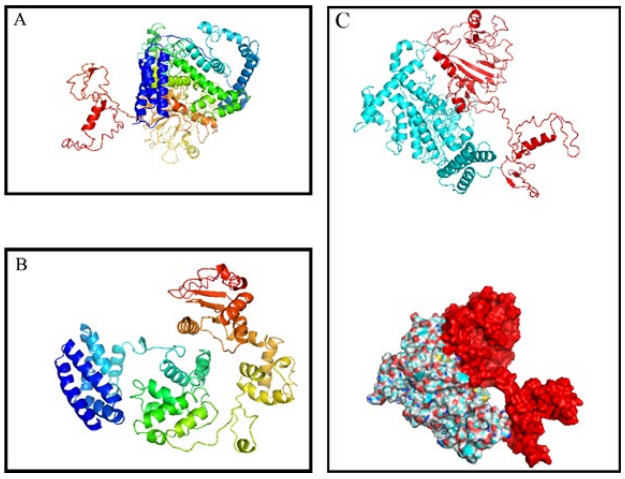

Figure 5. Structural Modeling of CAPN7
(A) Wild-type structure. (B) Mutant structure. (C) Deletion mapping; The deleted region is demarcated in red. The upper panel employs a cartoon representation to emphasize secondary structure elements (α-helices and β-sheets), while the lower panel provides a molecular surface view, revealing the three-dimensional conformational consequences of the truncation

The *CAPN7* c.1454G>A (p.Trp485Ter) nonsense variant in exon 13 introduces a premature termination codon, resulting in truncation of the catalytic cysteine protease domain, and is predicted to trigger nonsense-mediated mRNA decay (NMD) pathway. Based on ACMG criteria (PVS1, PM2, PP4, and PP1), the available evidence supports classifying this variant as pathogenic within the affected family. In addition, integrated computational assessment provides strong evidence of structural and functional disruption associated with the variant, importantly supported by transcriptomic data showing significantly decreased *CAPN7* expression in epilepsy patients. These findings, together with the observed segregation pattern, highlight the potential clinical relevance of this variant and underscore its importance in genetic counseling and risk assessment for affected families. Counseling should inform affected individuals and at-risk relatives about recurrence risks and preventive options, while also addressing current limitations in understanding the full clinical spectrum and long-term outcomes, given the novelty of this association, which emphasizes the need for further studies.

Previous studies have shown that persistent dysregulation of calpain is involved in both acute and chronic neurodegenerative processes across a range of pathological conditions, and its role in epileptogenesis has been widely proposed. However, since calpains belong to a large family of proteases, it has been challenging to identify the specific roles of individual isoforms in epilepsy (37, 38). In this context, the link between calpain genes and epilepsy is an emerging area of research.

While direct evidence remains limited, a case report documented a pediatric patient with a pathogenic *CAPN3* variant exhibiting both limb-girdle muscular dystrophy (LGMD) and GGE, suggesting a potential link between *CAPN3* variants and the development of hereditary

generalized epilepsy (39). Recently, a family carrying a *CAPN3* variant was reported, in which one homozygous individual exhibited the LGMD2A phenotype, while several heterozygous relatives, including three confirmed carriers, were diagnosed with GGE (40). Therefore, *CAPN3* variants may play a role in GGE. Given that *CAPN7* shares sequence homology with *CAPN3*, the causative gene for LGMD2A, it is conceivable that *CAPN7* might also be involved in epilepsy (41).

Additional evidence supporting the potential involvement of *CAPN7* in epilepsy arises from its proposed role in membrane trafficking, although current knowledge regarding this function remains limited. Notably, the MIT domains of CAPN7 have been reported to associate with selected components of the endosomal sorting complex required for transport (ESCRT)-III and related proteins, such as charged multivesicular body protein one and increased sodium tolerance 1 (IST1) (42). Based on the STRING Interaction Network, IST1 is one of the interacting proteins with CAPN7. Also, based on the International Mouse Phenotyping Consortium (IMPC), GGE is one of the predicted associated diseases with IST1.

The collective evidence and findings suggest a novel pathogenic mechanism potentially involving disruption of calpain-mediated proteolytic pathways within neuronal networks. The observed reduction in *CAPN7* expression in patients with epilepsy provides preliminary support for its role in epileptogenesis, although the precise downstream consequences remain to be elucidated. In the long term, clarifying *CAPN7*-related epileptogenic mechanisms may facilitate the development of targeted therapeutic strategies, including calpain-specific modulators and gene-based interventions.

Conclusion

This study reports, for the first time, a potential association between *CAPN7* and GGE, offering a novel perspective on its role in epilepsy pathogenesis. However, further functional studies and identification of additional families segregating GGE with *CAPN7* variants are needed to confirm this association. These findings could broaden diagnostic and therapeutic approaches for GGEs, supporting genetic counseling, family screening, and prevention, especially in populations with high consanguinity, and may facilitate the future development of targeted treatments.

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Authors' Contribution

F J contributed to study design, performed experiments, collected and processed data, conducted initial analyses, and drafted the manuscript. M S, MT F, and M F conducted clinical evaluations, participated in patient recruitment, and collected clinical data. MSK contributed to data analysis. J W, A SN, and M Z contributed to study design, data analysis, and interpretation of results, revised the manuscript, supervised the study, and acquired funding. All authors approved the final version to be published.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration

We acknowledge the use of ChatGPT to improve the language and readability.

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