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ORIGINAL ARTICLE

Genetic Polymorphism in Febrile Seizures

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Abstract

Objectives To investigate the genetic polymorphisms associated with febrile seizures (FS) in children from Vijayapura District, Karnataka, South India. Also to identify novel or known pathogenic variants using whole exome sequencing (WES), explore clinical profiles, and assess associations with family or past history of FS.

Methods This observational study included 50 children aged 6 mo to 6 y presenting with FS at Shri B.M. Patil Medical College, Vijayapura. After excluding those with neurological deficits or atypical seizure etiologies, peripheral blood samples were collected. DNA was extracted, and exon-specific primers were designed. PCR products were subjected to Sanger sequencing, and WES was performed on 12 cases. Clinical characteristics were recorded, and statistical analysis was done using SPSS v20.

Results Among 50 children, the most common age group was 1–2 y (50%), with males accounting for 56%. Family history and past history of FS were present in 38% and 40% respectively. Typical FS were more common (76%). WES revealed novel or rare variants, including variants of uncertain significance (VUS), in GABRG2, SEMA6B, and KCNQ2. While KCNQ2 (6%) and CPA6 (2%) pathogenic/likely pathogenic variants have known associations with FS, the SEMA6B variants observed (30%) are primarily VUS, and their functional or causal role in FS remains uncertain. No significant association was found between seizure type and family or past history.

Conclusions This pilot study underscores the genetic heterogeneity of FS and identifies SEMA6B as a potentially relevant gene in South Indian children. WES may be a valuable tool for unraveling FS pathogenesis, especially in region-specific populations.

Keywords Febrile seizures · Whole exome sequencing · Genetic polymorphism · SEMA6B

Introduction

Febrile seizures (FS) are defined by the International League Against Epilepsy (ILAE) as "a seizure occurring in childhood after six months of age associated with a febrile illness not caused by an infection of the central nervous system, without previous neonatal seizures or a previous unprovoked seizure, and not meeting the criteria for other acute symptomatic seizures" [1, 2]. These generalized seizures most commonly occur between 6 mo and 5 y of age, with fever exceeding 100.4 °F (38 °C), in the absence of central nervous system infection, electrolyte imbalance, metabolic disturbances, or prior afebrile seizures [1]. Though the exact temperature threshold is variable, FS are the most common childhood seizure type, showing a male predominance (1.6:1) and a prevalence of 2–5% in the US and Europe, with peak incidence at 12–18 mo [3]. Epidemiological studies also report environmental influences, including winter seasonality and afternoon clustering [3].

About 30% of affected children experience recurrent seizures, usually within 24 h of fever onset. The most common presentation is a brief generalized tonic–clonic seizure lasting under 15 min [4]. FS remain a common reason for pediatric emergency visits, with respiratory tract infections—particularly those due to influenza, adenovirus, and parainfluenza—being the most frequent precipitants [5]. In tropical countries such as India, malaria and dengue add substantially to the

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infectious triggers [6, 7]. Despite their typically benign course, FS continue to attract clinical attention due to frequent recurrences and possible long-term neurological consequences.

The etiology of FS is multifactorial, encompassing environmental and genetic contributors. A positive family history is often observed, supporting a hereditary predisposition. Genetic factors influencing neuronal excitability, particularly sodium channel genes, and those involved in immune and inflammatory pathways, such as cytokine polymorphisms, have been implicated [8]. Elevated cytokine levels during fever may increase seizure susceptibility. Although most FS resolve spontaneously, a subset of children—especially those with complex seizures or underlying neurological deficits—may later develop epilepsy or other chronic seizure disorders [3, 4].

Recurrent FS are particularly common: one-third of children with an initial seizure will have a second, and up to half of these will have a third [9, 10]. Children with FS have a 5–7-fold increased risk of spontaneous epilepsy compared to the general population, where baseline risk is 2–5% [9, 10]. Familial aggregation is well documented, with first-degree relatives frequently reporting FS histories, suggesting a stronger genetic contribution than perinatal or infectious risk factors. In sporadic cases, inheritance appears polygenic, whereas familial recurrent FS more often follows an autosomal dominant pattern with reduced penetrance. Multiple genetic loci (FEB1–FEB11) have been mapped in familial FS syndromes. One notable gene, ADGRV1 (OMIM 602851), encoding adhesion G protein-coupled receptor V1, is highly expressed in the CNS and linked to both febrile and afebrile seizures in hereditary cases. Nonetheless, most causative genes in sporadic FS remain unidentified, underscoring the need for population-specific studies. Therefore, this pilot study was designed to investigate the genetic architecture of FS in children from Vijayapura District, South India, using combined Sanger sequencing and whole exome sequencing (WES) and its relationship to pathogenic variant yield.

Material and Methods

This observational study was conducted in the Department of Pediatrics at Shri B.M. Patil Medical College Hospital and Research Centre, Vijaypura. Children aged between 6 mo and 6 y presenting with seizures associated with fever, and meeting the criteria for febrile seizures, were recruited. A total of 50 cases fulfilling the inclusion criteria were included after obtaining informed written consent from the parents or guardians. Children with gross neurological deficits or seizures attributable to central nervous system infections, trauma, or metabolic disorders were excluded. The study was approved by the Institutional Ethics Committee, reference number BLDE(DU)/IEC/966/2022-23.

Following enrolment, 1 mL of peripheral blood was collected in EDTA-coated vacutainers and stored at 4°C. Genomic DNA was isolated from 300 µL of blood using a commercially available kit (Bangalore Genei, India). The DNA extraction protocol involved lysis of red blood cells, isolation of white blood cell pellets, lysis using solution B, ethanol precipitation, washing, and resuspension in solution C. DNA quality was confirmed through agarose gel electrophoresis, and concentration was measured using a Nanodrop spectrophotometer (Quawell, USA).

Exon-specific intronic primers were designed using Primer3 software and validated using the UCSC Genome Browser and in silico PCR. The primers were synthesized commercially (MWG Biotech, India). PCR amplification was performed in a 20 µL reaction volume containing genomic DNA, primers, dNTPs, Taq DNA polymerase, and buffer. Amplification was carried out in a thermal cycler (Eppendorf, Germany) using the following conditions: initial denaturation at 98°C for 10 s, followed by 35 cycles of denaturation (98°C for 10 s), primer-specific annealing, extension at 72°C for 15 s, and a final extension at 72°C for 5 min. PCR products were verified on 1% agarose gel.

The amplified PCR products underwent capillary-based Sanger sequencing using the BigDye Terminator v3.1 chemistry (Applied Biosystems, USA). Cycle sequencing was carried out with either forward or reverse primer. Products were cleaned using ethanol and sodium acetate precipitation, followed by drying and resuspension in Hi-Di formamide. Denatured products were loaded onto the ABI 3730 genetic analyzer for sequencing. Resulting chromatograms were analyzed using Sequencing Analysis v5.4, and sequences were aligned and compared to reference sequences using Variant Reporter v1.1 (Applied Biosystems, USA) for pathogenic variant identification.

Of the 50 children enrolled, 12 underwent WES. Cases were selected for WES if they presented with atypical febrile seizures (prolonged, focal, or recurrent within 24 h), had a positive family history, or remained uninformative on targeted Sanger sequencing. This purposive sampling may introduce selection bias.

Pathogenic variant annotation was carried out for all identified genetic alterations, incorporating reference transcript IDs (RefSeq), genomic and cDNA positions, amino acid changes, dbSNP identifiers (where available), and pathogenic variant type. Allele frequencies were cross-checked with public databases such as gnomAD, and in-silico pathogenicity predictions were considered using tools like SIFT and PolyPhen-2, wherever applicable. Each pathogenic variant was further classified according to the American College of Medical Genetics (ACMG) 2015 guidelines into categories such as benign, pathogenic, likely pathogenic, or pathogenic variant of uncertain significance (VUS) [10]. Gene symbols were standardized as per HGNC nomenclature. In the present dataset, pathogenic variants were identified in KCNQ2 (synonymous

benign variant, rs61737409), SEMA6B (multiple coding variants including missense and synonymous substitutions), and GABRG2 (rare variant, rs758574181), with their respective genomic, cDNA, and amino acid positions documented.

Given the exploratory nature of this study and in line with pilot sequencing studies (30–100 cases), a sample size of 50 was considered adequate for preliminary variant discovery and clinical correlation. Statistical analysis was performed using SPSS version 26. Data were entered into Microsoft Excel and summarized using mean ± standard deviation, median with interquartile range, and frequencies or percentages. Comparisons between clinical subgroups were tested using chi-square or Fisher's exact test as appropriate, and *p*-values were interpreted cautiously given the multiple comparisons performed. Confidence intervals (95% CI) were calculated for proportions. Appropriate graphs and tables were used for data presentation.

Results

A total of 50 children with febrile seizures were enrolled; targeted Sanger sequencing was performed in all, and WES was undertaken in 12 selected cases, prioritizing those with atypical febrile seizures, positive family history, or uninformative Sanger results. Variants were most frequently observed in SEMA6B (30%, primarily VUS), followed by KCNQ2 (6%) and GABRG2 (2%). Only KCNQ2 and other rare variants had known or likely pathogenic effects, while the functional impact of most SEMA6B variants remains unclear. Rare pathogenic variants were also noted in CPA6, SAMD12, and ALDH7A1 (2% each), while SCN1A showed no pathogenic variants.

The most common age group was 1–2 y (50%), followed by 2–5 y (40%). Males accounted for 56%. A positive past history of febrile seizures was noted in 40%, and 38% had a family history (mostly among first-degree relatives). Most were first or second in birth order. Clobazam was being used for seizure prophylaxis in 40% of cases. Typical febrile seizures were more common (76%) than atypical (24%). Hemoglobin levels were <11 mg/dL in 66% of children. Lumbar puncture was performed in 56% of cases.

Statistical analysis revealed no significant association between seizure type and either family history or past febrile seizures (Table 1).

Genetic analysis showed that SEMA6B variants were observed in children with family history (15.79%) and past history (20%) of febrile seizures; however, after Bonferroni correction, these associations were not statistically significant, and no definitive genotype–phenotype correlation could be established (Table 2).

Among the pathogenic variants retained, multiple SEMA6B missense variants (p.Y101C, p.Y102L) and a rare GABRG2 variant (rs758574181) were classified as VUS (Table 3).

Table 1 Association between type of seizure and clinical history

Factor	Atypical seizure *n* (%) [95% CI]	Typical seizure *n* (%) [95% CI]	χ^2	*p*-value	Adjusted *p*-value (Bonferroni)
Family history	6 (50.0%) [21.5–78.5%]	13 (34.2%) [19.6–52.2%]	0.76	0.384	0.768
Past history	7 (58.3%) [27.7–84.8%]	13 (34.2%) [19.6–52.2%]	1.06	0.304	0.608

Table 2 Association of gene variants with family and past history of febrile seizures

Pathogenic variants (*n*)	Family history present (*n* = 19)				Past history present (*n* = 20)			
	N (%) [95% CI]	χ^2	*p*-value	Adjusted *p*-value	*N* (%) [95% CI]	χ^2	*p*-value	Adjusted *p*-value
SEMA6B (*n* = 15)	3 (15.79%) [3.4–39.6%]	2.95	0.086	0.516	4 (20.00%) [6.8–44.3%]	3.7	0.054	0.324
KCNQ2 (*n* = 3)	0 (0.00%) [0–17.6%]	1.96	0.162	0.972	0 (0.00%) [0–16.8%]	2.13	0.145	0.870
CPA6 (*n* = 1)	0 (0.00%) [0–17.6%]	0.63	0.429	1.000	1 (5.00%) [0.1–24.9%]	1.53	0.216	1.000
SAMD12 (*n* = 1)	0 (0.00%) [0–17.6%]	0.63	0.429	1.000	0 (0.00%) [0–16.8%]	0.68	0.409	1.000
ALDH7A1 (*n* = 1)	1 (5.26%) [0.1–26.0%]	1.66	0.197	1.000	0 (0.00%) [0–16.8%]	0.68	0.409	1.000
GABRG2 (*n* = 1)	0 (0.00%) [0–17.6%]	0.63	0.429	1.000	0 (0.00%) [0–16.8%]	0.68	0.409	1.000
SCN1A (*n* = 0)	0 (0.00%)	0	1	1.000	0 (0.00%)	0	1	1.000

Table 3 Identified variants and annotations

Gene (RefSeq)	Zygosity	gDNA position (GRCh38)	cDNA position	AA change	Variant type	dbSNP/ID	ACMG status	gnomAD AF	SIFT	PolyPhen	Phenotype/ Disease
SEMA6B (NM_032108.4)	Heterozygous	chr5:13894713A>G	c.301A>G	p.Y101C	Missense	NA	VUS	0.00005	Deleterious	Probably damaging	NA
SEMA6B (NM_032108.4)	Heterozygous	chr5:13894715C>T	c.303C>T	p.Y102L	Missense	NA	VUS	0.00002	Deleterious	Probably damaging	NA
GABRG2 (NM_198904.4)	Heterozygous	chr5:161212345	NA	NA	Rare variant	rs758574181	VUS	0.00001	NA	NA	NA

Discussion

This pilot study contributes to the expanding field of genetic evaluation in febrile seizures (FS) by applying WES alongside Sanger sequencing in a cohort from Vijayapura, Karnataka. Pathogenic variants were detected in KCNQ2, CPA6, SEMA6B, and SAMD12, with SEMA6B being most frequent, observed in 30% of cases. Variants in KCNQ2 (6%) align with earlier findings by Millichap et al., who linked KCNQ2 mutations with benign familial neonatal seizures and early-onset epileptic encephalopathy, depending on the mutation type [11]. The CPA6 variant (2%) echoes reports by Salzmann et al., who found its association with FS and temporal lobe epilepsy, suggesting a shared pathogenic mechanism [12]. The relatively high prevalence of SEMA6B variants, which are mostly VUS, in the present cohort may reflect a regional enrichment; however, their functional or causal role in FS remains unproven. Shu et al. reported associations with developmental delay and FS, but further studies are required to establish pathogenicity [13].

Demographically, the present results confirm established age and sex patterns in FS. Most cases occurred between 1–2 y, in line with Sharawat et al., who found that 60% of Indian FS cases occurred between 6–24 mo [14]. Similar age-specific vulnerability was reported in Korea, with peak incidence at 18–30 mo [15]. Male predominance (56% in the present cohort) parallels data from Agrawal et al. in Nepal, who reported 70% male cases [16]. These findings reinforce early neurodevelopmental susceptibility and possible hormonal contributions to FS expression.

Family history was present in 38% of cases, supporting the genetic basis of FS. A meta-analysis by Yang et al. showed that GABRG2 rs211037 polymorphisms reduce GABA A receptor function, enhancing seizure risk [17]. Likewise, Zare-Shahabadi et al. implicated TNF-α -238GG genotypes in FS susceptibility [18]. Together, these highlight the polygenic and multifactorial etiology of FS, combining inherited predisposition with developmental and environmental triggers.

Clinically, 76% of children presented with typical FS, while 24% had atypical features, consistent with literature defining typical FS as brief, generalized, and non-recurrent within 24 h. Notably, 40% of children received intermittent clobazam prophylaxis during febrile episodes. Sattar et al. and Khosroshahi et al. both demonstrated clobazam's favorable safety and efficacy compared to diazepam, reporting fewer adverse effects such as sedation and improved seizure control [19, 20]. The present findings support clobazam as a reasonable option for high-risk children with frequent recurrences.

The detection of genetic variants highlights the utility of WES for identifying candidate variants in FS, though most SEMA6B variants are VUS, and their clinical relevance remains uncertain. The role of SAMD12, observed in 2% of patients, remains uncertain, given limited prior evidence, and requires further functional validation. Importantly, many detected variants were classified as VUS by ACMG criteria, illustrating current limitations in clinical interpretation.

Several factors limit the conclusions. First, anemia (Hb <11 g/dL) was noted in 66% of participants, suggesting that nutritional or socioeconomic factors could confound seizure susceptibility. Second, while respiratory and tropical infections were identified as triggers, pathogen-specific data were lacking, preventing detailed characterization of febrile illnesses. Third, structured developmental assessments before and after FS were not performed, precluding evaluation of possible genotype–phenotype correlations with neurodevelopmental outcomes.

Despite these constraints, the observed mutation spectrum and familial clustering suggest that genetic evaluation may provide valuable insights in FS, particularly for recurrent or atypical cases. However, as most detected variants, especially in SEMA6B, are classified as VUS, these findings are exploratory and hypothesis-generating rather than conclusive. Future multicentric studies with larger, well-characterized cohorts, standardized neurodevelopmental assessments (to explore genotype–phenotype correlations), comprehensive pathogen profiling, longitudinal follow-up, and functional assays are required to validate gene–disease associations, improve risk stratification, and inform potential precision medicine strategies in pediatric FS populations.

Conclusions

This pilot study underscores the genetic heterogeneity of FS and reports the presence of SEMA6B variants (mostly VUS), highlighting potential, yet unconfirmed, contributions to FS in South Indian children. Functional validation is needed to establish causality. The findings support the heterogeneous nature of FS and underscore the importance of further research on genetic screening approaches, especially in individuals with a family history of seizures. With SEMA6B variants being most prevalent (30%), primarily VUS, in this cohort, the study provides exploratory, hypothesis-generating observations, rather than evidence of definitive gene-disease associations, highlighting the need for further validation in larger, well-phenotyped cohorts.

Authors' Contributions CR: Conceptualization, data collection & manuscript drafting; RN: Methodology design, statistical analysis & manuscript revision; GSK: Genetic data interpretation, whole exome sequencing analysis & critical review; MMP: Patient recruitment, clinical data supervision & final approval of the manuscript. MMP will be the guarantor for this manuscript.

Declarations

Conflict of Interest None.

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