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SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES

DR CHINMAYA RODGI

PEDIATRICS PG

UNDER THE GUIDANCE OF:

DR RAVINDRA NAGANOOR

PROFESSOR

DEPT OF PEDIATRICS

CO GUIDE :

DR G S KADAKOL

RESEARCH SCIENTIST

HUMAN GENETICS LABORATORY

DEPARTMENT OF ANATOMY

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled " **THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES**" has been prepared by me under the supervision and guidance of **DR RAVINDRA NAGANOOR, PROFESSOR**

DEPT OF PEDIATRICS. This is being submitted to BLDE (Deemed to be University) Shri. B. M. Patil Medical College, Hospital & RC, Vijayapura, Karnataka in partial fulfilment of the requirement for the award of a master's degree in Pediatrics. This work has not been submitted to any University by me for the award of any degree.

Date:

Place: Vijayapura.

DR CHINMAYA RODGI

Post Graduate Student,

Department of Pediatrics,

B.L.D.E. (DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College,

Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE GUIDE & CO-GUIDE

This is to certify that the dissertation entitled " **THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES**" is a bonafide and genuine research work carried out by Dr. **CHINMAYA RODGI** in partial fulfilment of the requirement for the degree of Doctor of Medicine in Pediatrics.

Date:

Place: Vijayapura

DR. RAVINDRA NAGANOOR
MD PAEDIATRICS
PROFESSOR
DEPARTMENT OF PAEDIATRICS
B.L.D.E(DEEMED TO BE UNIVERSITY)

DR. G.S. KADAKOL
Research scientist, SAST
ASSISTANT PROFESSOR
DEPARTMENT OF ANATOMY
B.L.D.E(DEEMED TO BE UNIVERSITY)

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that the dissertation entitled " **THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES** " is a bonafide research work done by Dr. **CHINMAYA RODGI** under the guidance of **DR RAVINDRA NAGANOOR**

Professor, Department of Pediatrics, Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.

Date:

Place: Vijayapura

DR M. M. PATIL

M.D.PEDIATRICS

Professor and HOD

DEPT OF PEDIATRICS

B.L.D.E. (DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College,

Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY THE PRINCIPAL

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Date:

Place: Vijayapura

Dr. ARAVIND PATIL
PRINCIPAL
B.L.D.E. (DEEMED TO BE UNIVERSITY)
Shri B. M. Patil Medical College,
Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

I hereby declare that the B.L.D.E. (DEEMED TO BE UNIVERSITY), VIJAYAPURA, Karnataka shall have the rights to preserve, use, and disseminate this dissertation/thesis in print or electronic format for academic/research purposes.

Date:

Place: Vijayapura

DR. CHINMAYA RODGI

Post Graduate Student,

Department of Pediatrics,

B.L.D.E.(DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical, College,

Hospital & Research Centre, Vijayapura

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

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Date: Signature of the Candidate

Place: Vijayapura Name: **Dr. Chinmaya Rodgi**

ABSTRACT

INTRODUCTION

"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" is defined as a febrile seizure. The most prevalent kind of seizures in children are febrile seizures, which have a 1.6:1 male predominance. Compared to the general population who experience FSs, children with a family history of FS have a three-fold or higher risk of recurrence. Recurrent FSs are thought to be more compatible with a positive family history among first-degree relatives than with recent infections, fever, and perinatal exposure. Numerous studies have so far identified the genetic heterogeneity of familial FSs (referred to as FEB1–FEB11). Specifically, ADGRV1 (OMIM *602851), which genes for the large calcium-binding protein adhesion G protein-coupled receptor (aGPCR) V1, which is abundantly expressed in the central nervous system, was previously discovered as a hereditary cause of afebrile and febrile seizures.

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AIMS OF THE STUDY

To study the genes associated with febrile seizures and genetic susceptibility in Vijayapura region of Karnataka, South India.

OBJECTIVES OF THE STUDY

To assess the role of the genes in genetic predisposition to the development of febrile convulsions.

Materials and methods

The study would cover all cases of fever-related seizures admitted to the Paediatrics Department at Shri B.M. Patil Medical College Hospital and Research Centre in Vijayapura that match the inclusion and exclusion criteria. A minimum of 50 instances will be examined. An incident that occurs in infancy or childhood, typically between the ages of 6 months and 6 years, and is characterised by fever but no indication of intracranial infection. The subjects enrolled in the study provided consent. Following consent, 1 ml of peripheral blood was collected in EDTA-coated vacutainers (BD367863) and stored at 4°C. PCR products were

sequenced using capillary-based Big-Dye terminators. Prior to sequencing, the PCR products went through cycle sequencing and plate processing.

Statistical analysis:

The collected data will be loaded into a Microsoft Excel spreadsheet, and statistical analysis will be carried out using the statistical programme for the social sciences (Version 20).

The results will be reported as mean ± SD, median, interquartile range, frequency, percentages and graphs.

Results:

We found that majority were in 1-2 years i.e50%(n-25) followed by 2-5 years 40%(n-20) and least in >12 months of age, 56%(n-28) are males and 44%(n-22) were Females . 38% (n-19) had family history of febrile Convulsion and 62%(n-31) had no family history of febrile Convulsion, 76%(n-38) had typical febrile seizures and 24%(n-12) had atypical febrile seizures. KCNQ2 gene is positive in 6.% (n-3) patients

Conclusion

The extensive genetic diversity within FS requires patients to undergo complete genetic testing because researchers have proven its necessity through their examination. Research indicates that genetic mutations found in KCNQ2, CPA6 and SEMA6B genes affect susceptibility to FS in patients. More extensive research involving bigger participant groups should occur to both confirm these gene association findings and explain how these mutations cause FS.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

AA	arachidonic acid
BBB	blood-brain barrier
CNS	central nervous system
COX2	cyclooxygenase-2
ILAE	International League Against Epilepsy
GABRG2	Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma-2.
GEFS +	generalized epilepsy with febrile seizure plus
GAA	gamma-aminobutyric acid
FEB	Febrile Epilepsy
FS	febrile seizures
KCNC	Potassium Voltage-Gated Channel,
LPS	Lipopolysaccharide
LP	Lumbar puncture
SCN1A	Sodium Voltage-Gated Channel Alpha Subunit 1.
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor.

THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES

INTRODUCTION:

"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" is defined as a febrile seizure by the International League Against Epilepsy (ILAE).[1, 2], Generalized seizures known as febrile seizures usually affect children aged 6 months to 5 years. They are distinguished by a fever surpassing 100.4 °F (38 °C) and are not associated with a central nervous system (CNS) illness, a known seizure-inducing cause (such as electrolyte imbalance, hypoglycaemia, or substance addiction), or a history of afebrile seizures. [1.] There is no fixed threshold value for fever to produce febrile seizures because each patient's convulsive temperature is different.

The most prevalent kind of seizures in children are febrile seizures, which have a 1.6:1 male predominance. The prevalence of febrile seizures in children in the US and Europe ranges from 2% to 5%, peaking between the ages of 12 and 18 months. In the US, Finland, and Japan, a seasonal and diurnal relationship has also been noted, with more episodes taking place in the winter and afternoon.[3]. Thirty per cent of young children experience many febrile seizures, whereas some only experience one.

Most febrile seizures (FSs) happen within 24 hours after the commencement of fever, though fever can happen at any moment during or after a seizure. Fever accompanied by isolated, widespread tonic-clonic seizures that don't happen within 24 hours and last less than 15 minutes.[4]

In emergency and outpatient departments, febrile seizures are among the most prevalent neurological symptoms. Respiratory tract infection is the infection most frequently linked to FS. Children with FS are most frequently infected with the influenza virus, adenovirus, and parainfluenza virus [5]. Malaria and dengue are two tropical illnesses that are major contributors to febrile seizures in India. Over the past ten years, there have been significant advancements in our knowledge of FS. [6, 7].

It appears that both hereditary and environmental variables play a role in the multifactorial aetiology of FS. There is frequently a familial history of FS in these patients. Genes linked to neuronal excitability, including ion channels, especially sodium channels, and genes involved in the immunological inflammatory response are examples of genetic influences.

An increased chance of having FS has been linked to increased levels of inflammatory molecules, such as cytokines. Environmental factors, mostly related with specific viral triggers, and genetic variations in cytokine genes are factors that regulate the inflammatory response..[8].

The majority of febrile seizures end on their own without any consequences. However, there is evidence from studies that after having a febrile seizure, some patients may be more likely to develop epilepsy or another seizure disorder. According to some specialists, patients are predisposed to a seizure disease by either an underlying neurologic problem or the impact of a febrile seizure on a growing nervous system.[3, 4] . Variations in some susceptibility genes appear to contribute to the genetic complexity of simple FS diseases. [11].

Fifty percent of patients who have experienced FS will encounter a third episode, and around one-third will experience a second attack. 5 to 15% of epileptic patients have a history of FS from childhood, and children under the age of 14 who have complicated FS before them are more likely to develop epilepsy. Children with FS have a 5–7 times higher risk of spontaneous seizures than the general population, which is estimated to be 2–5%. [9, 10]..

Children who have a close relative with familial spasticity face a three-fold or greater possibility of developing the condition again. Studies indicate that FSs which recur tend to show positive results in first-degree relative history tests instead of tests based on recent infections or fever or perinatal exposure [9, 10]. Research indicated a polygenic model in probands who experienced one FS but autosomal dominancy with reduced penetrance stood as the best explanation for multiple FSs in family studies. Research has established multiple distinct genetic causes of familial FSs which have been named from FEB1 to FEB11. ADGRV1 (OMIM *602851) serves as the genetic origin for aGPCR V1 that shows high expression throughout the central nervous system thus leading to hereditary cases of febrile and afebrile seizures.

Numerous genetic loci for FSs have lately been found, although particular genes affecting the bulk of FS cases have not yet been identified. Genetic factors are known to play a significant influence in the vulnerability to febrile seizures, and the condition is complex and heterogeneous.

This study aims to identify the range of genes linked to febrile seizures in the community in and around the Vijayapura District of Karnataka, South India.

There is a need for this research due to the high occurrence of this condition and very little evidence of studies on the genetic polymorphism of febrile convulsions.

AIMS OF THE STUDY

To study the genes associated with febrile seizures and genetic susceptibility in the Vijayapura region of Karnataka, South India.

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To assess the role of the genes in genetic predisposition to the development of febrile convulsions

REVIEW OF LITERATURE

The onset of febrile seizures affects young children from 6 months to 5 years who experience generalized febrile convulsions beyond 100.4 °F (38 °C) that are unaffected by CNS infections or known seizure triggers including electrolyte disturbances and substance misuse or previous episodes of afebrile seizures. The convulsive temperature thresholds differ between patients so medical experts cannot establish an exact fever threshold which produces febrile seizures. Professionals identify both neurologic impairment and viral infection presence along with hereditary seizures as well as developmental delays and blood zinc and iron deficiencies, maternal smoking habits and stress as the principal causes of febrile seizures.

The majority of febrile seizures end on their own without any consequences. However, there is evidence from studies that after having a febrile seizure, some patients may be more likely to develop epilepsy or another seizure disease. Since the majority of febrile seizures end on their own, expectant management is possible. Pharmacologic treatment, however, can be necessary to halt the seizure activity in complex or prolonged febrile seizures.[3].

Epidemiology

The most common neurologic disorder in children is febrile seizures, which afflict 2-5% of children in the United States and Western Europe aged 6 months to 5 years, peaking between the ages of 12 and 18 months. All ethnic groups get febrile seizures, however, Asians are more prone to have them (5-10% of Indian children and 6-9% of Japanese children). Children from lower socioeconomic backgrounds are more likely to have the disorder, most likely as a result of limited access to healthcare. Researchers in the United States have noted seasonal and diurnal fluctuations in the incidence of febrile seizures. Japan and Finland. In general, febrile

seizures tend to happen in the afternoon and during the winter.[12,13.] Gender does not appear to have an effect on incidence. There have been cases of children as young as three months old and as old as seven years old having their first febrile seizure, with the majority occurring between the ages of six months and five years. Children aged 12 to 18 months have the highest incidence. In the winter, the prevalence of febrile infections in young children soars..[14]

Pathophysiology

How does fever generate seizures?[15]

The development of febrile seizures depends on both hereditary and environmental elements while sodium channel and GABAA receptor and interleukin genes show primary influence. Alterations in brain temperature affect ion channels which then cause neurons to become more excitable and more prone to seizures. Studies prove that the developing brain becomes more sensitive to temperature fluctuations thus hyperthermia acts as a single cause of seizures in young children. Interleukin-1β produced by fever elevates neuronal excitability while it activates both glutamate and GABA neurotransmitter systems thus promoting seizure risk.

Body temperatures increase due to infections in the ear and throat space which cause inflammation leading to febrile seizures. Pro-inflammatory cytokine release occurs in immune cells after bacterial PAMPs activate TLRs on their surface. Blood-brain barrier failure together with increased cytokine levels and disrupted CNS operations occur when inflammation remains unchecked. The excessive amounts of LPS along with cytokines cause damage to central nervous system pathways while decreasing acetylcholine signalling effects and destroying blood-brain barrier protective structures.

The BBB becomes disrupted through Gram-negative bacterial LPS activation of TLR4 which subsequently triggers cytokine reactions and causes fever. Hyperthermia caused by fever

weakens the blood-brain barrier by affecting the connections between endothelial cells. The disorders result in functional disturbances and morphological alterations in astrocytic cells. LPS together with cytokines control P-gp transporter expression levels which leads to functional changes at the BBB.

Ill-1β modifies synaptic processes in hippocampus and CNS tissues resulting in changes that affect both ANS functions and the enteric nervous system. The IL-1RI receptor shows enhanced affinity for the substance which decreases the available IL-1Ra concentration and disrupts glutamatergic and GABAergic neuronal mechanisms. The function of GABA\(_A\) receptors becomes disrupted by IL-1β which reduces inhibitory currents and causes ion balance modification. Such neuronal excitability enhancement contributes to the development of febrile seizures.

Fever.[16,17,18]

Astrocytes, oligodendrocytes, and microglia are the three primary glial cell types found in the central nervous system (CNS), and each one has a distinct biological function. Microglia are the primary cells engaged in conquering infection in the central nervous system. The microglia are activated by cytokines that have crossed the blood-brain barrier. The CNS's resting microglia are distinguished by their tiny cell bodies with intricate, thin processes dispersed in various directions.[42]

When activated microglia retract their processes, their cell bodies enlarge, and their processes become thicker and fewer.[42] TNF-α, IL-1β, and IL-6 are among the cytokines that are produced and released by activated microglia.

Arachidonic acid (AA), a lipid produced from membrane phospholipids, is converted to PGE2 by the enzyme phospholipase A2. 46 When cyclooxygenase-2 (COX-2), an enzyme

expressed on brain endothelial cells in the hypothalamic preoptic area, is activated and AA is produced. COX-2 catalyses the manufacture of prostaglandins by oxidising AA and producing PGE2. Thermoregulatory neurones in the hypothalamic median preoptic nucleus then create EP3 prostaglandin receptors, which PGE2 binds to to produce a fever.

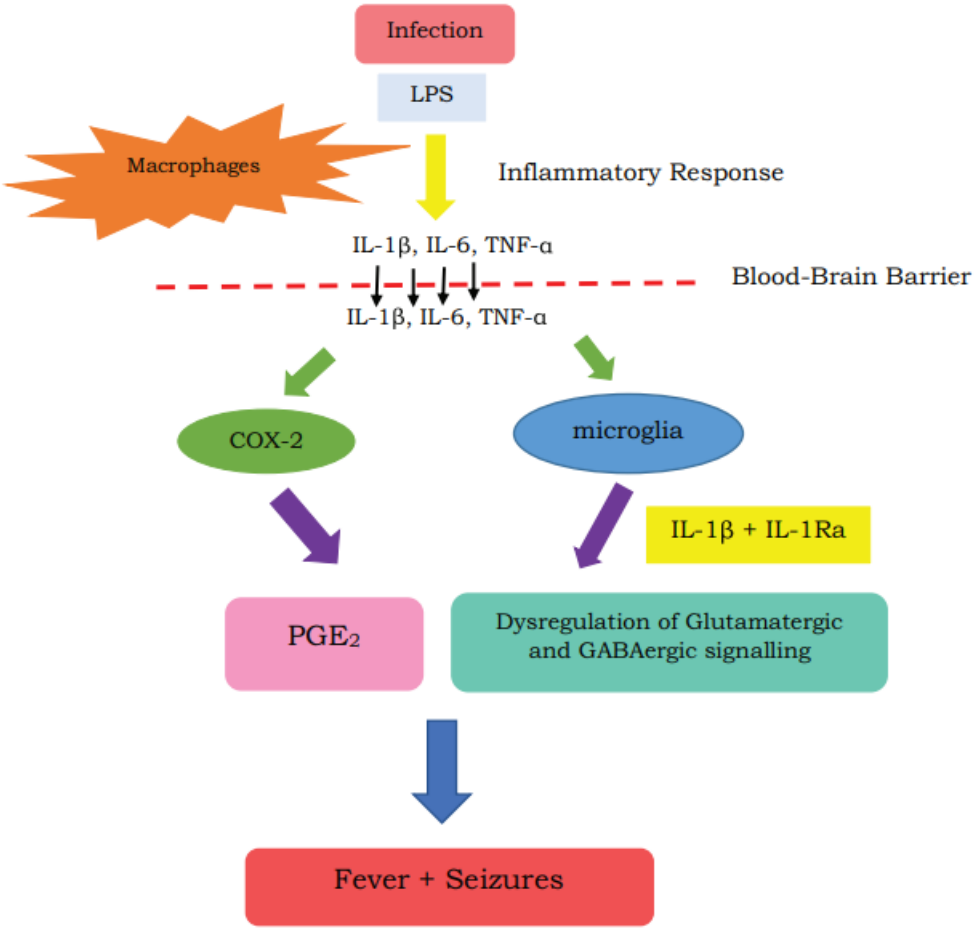


Figure 1 : A diagram depicting the pathogenesis of febrile seizures:

Etiology[5,19,20,21]

1. Age

Between the ages of six months and five, when the brain is still developing and has limited modulation skills and high excitability, febrile seizures are most common in children. Because every patient has a different convulsive temperature threshold, no set threshold for fever can cause febrile seizures. The presence of neurologic impairment, viral infection, family history of seizures, low blood zinc and iron levels[19], maternal smoking, and stress seem to be the main risk factors for febrile seizures.

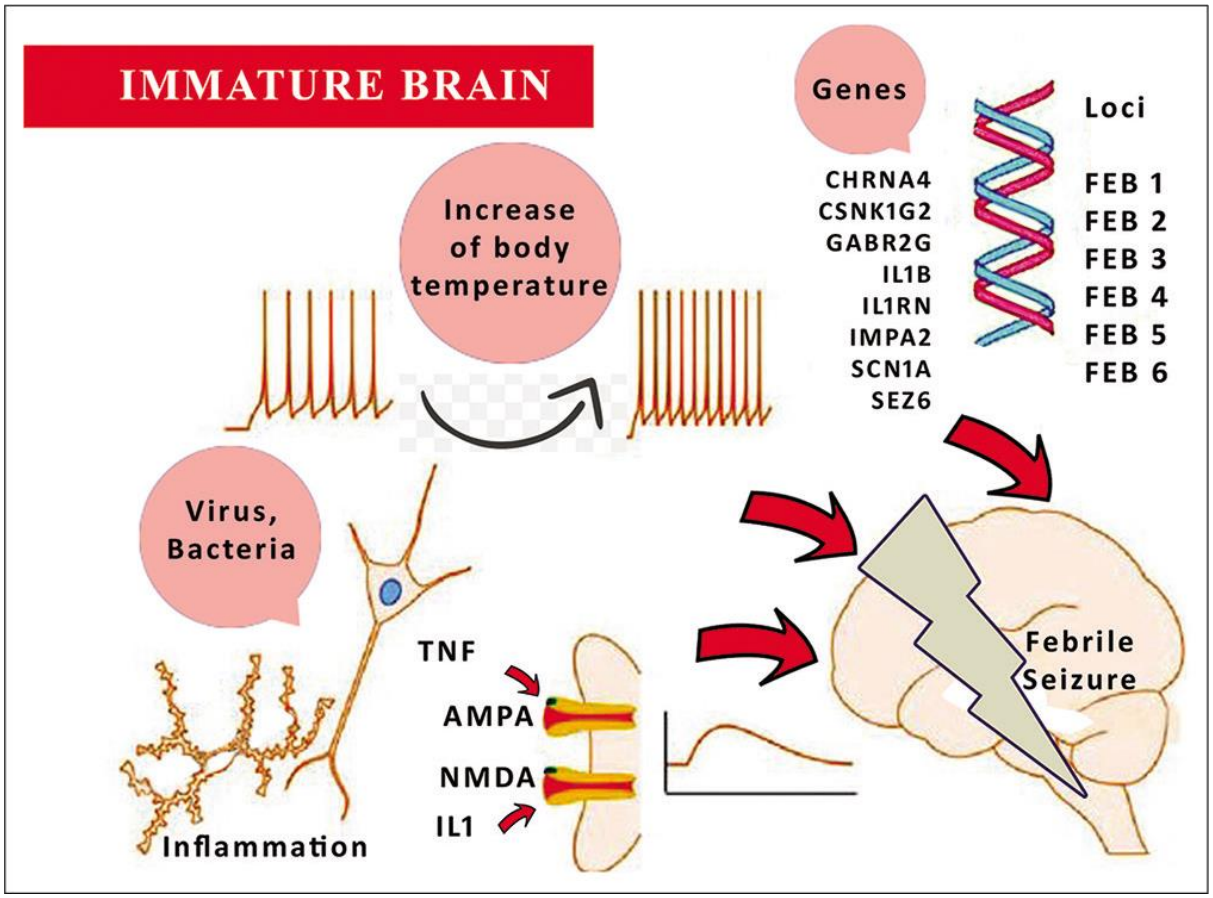


Figure 2:Genetic changes in febrile seizure

2. Fever: The primary sign is how quickly the body temperature rises, usually due to another illness. The onset of seizures is linked to hypothalamic temperature regulation and its effect on the seizure threshold, which varies from person to person and changes with age and growth. The highest temperature reached during a fever, rather than the rate of increase, may also influence the risk of febrile seizures.

3. Genetic Predisposition[9]: The majority of those impacted have a family history of epilepsy or febrile seizures. A concordance rate of around 35% to 69% in monozygotic twins and 14% to 20% in dizygotic twins5,20, as well as the estimated 10% to 33% of patients who have a first-degree relative with a positive seizure history, suggest that febrile seizures may have a genetic component. The following chromosome sites contain some of the genes that increase the risk of febrile seizures. There may also be a polygenic or multifactorial mode of inheritance, as well as an autosomal dominant form of heredity with reduced penetrance: 18p11*2, 19p13*3, 19q, 21q22, 5q14*15, 5q34, 6q22*24, 8q13*21, 2q23*34, 3p24.2*23, 3q26.2*26.33, and 5q31*15 have all been identified.

4. Neuroinflammatory Response and Neurotransmitter Imbalance: Cytokines, such as IL-1, TNF-α, and IL-6, are released during infections and have an impact on synaptic plasticity, neuronal excitability, and seizure threshold. The structures become more excitable, and the glutamate and GABA circuits become nervous. By altering GABA receptor function and ion channel activity, the genesis of such alterations in an inflammatory environment increases the likelihood of seizures.

5. Environmental Factors: Though 80% of viral illnesses rather than bacterial infections are more frequently linked to febrile seizures, there is no particular fever aetiology that is more likely to produce them. Up to one-third of infants under the age of two are found to have the roseola virus, the most prevalent virus linked to febrile seizures in the US and Europe. According to other research, complicated characteristics, recurrence, and febrile status epilepticus are more common in febrile seizures caused by the Roseola virus. However, influenza A has often been linked to febrile seizures in Asian countries. [22]

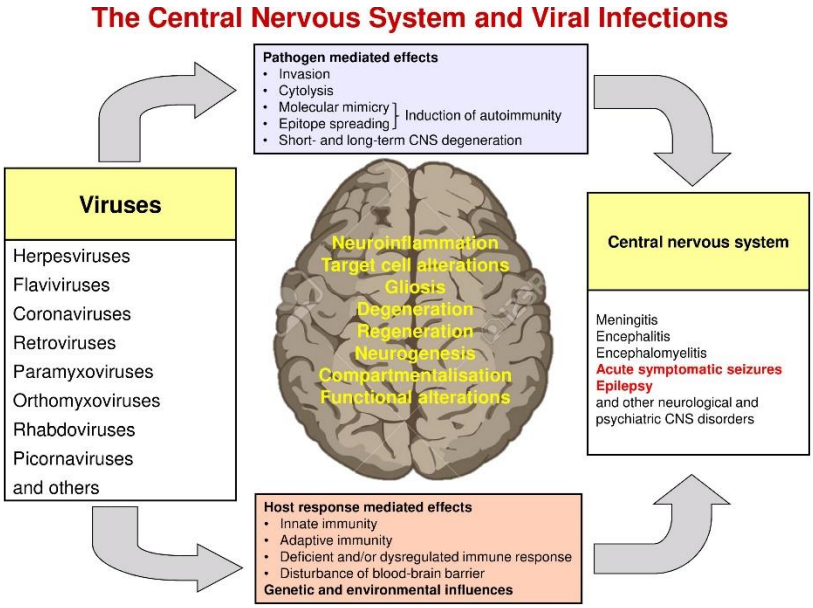


Figure 3: Etiology of febrile seizure

6. Additional factors: These include conjugated pneumococcal vaccine, MMRV, DTaP-IPV-Hib, and certain inactivated influenza vaccine formulations. According to a study, when IIV3, PCV, and DTaP-containing vaccinations were administered simultaneously, the greatest estimated absolute extra risk was 30 febrile seizures per 100,000 vaccinated individuals, as opposed to when they were administered on different days.

Although febrile seizures are mostly benign, they can recur, especially in children who have a genetic predisposition or those who have had a persistent fever.

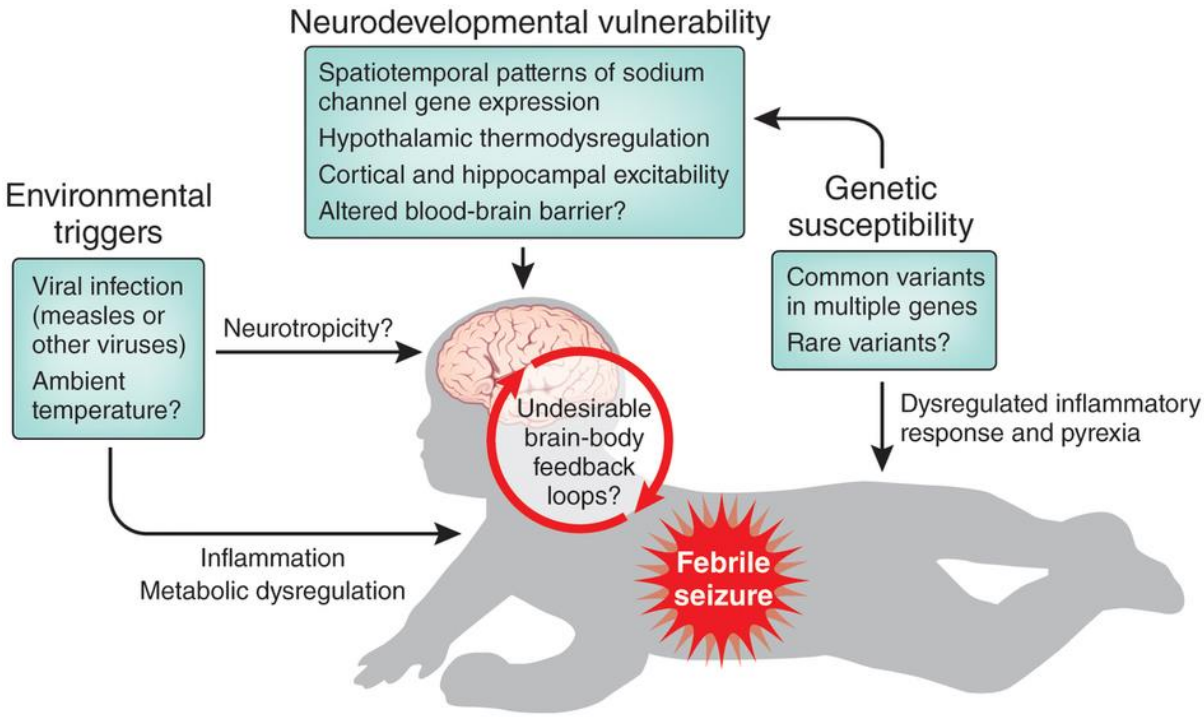


Figure 4: Aetiology of Febrile convulsions

Clinical manifestation [12,23]

Usually within an hour of the onset of a fever, febrile seizures happen during the first twenty-four hours of illness. In 25 to 50 percent of patients, the seizure is the first sign of a fever. 20 With an average duration of 4 to 7 minutes, only 10% to 15% of febrile seizures last longer than 10 minutes. One study found that patients typically had high temperatures, averaging 39.4°C. Frequent symptoms of febrile seizures include breathing difficulties, pallor or cyanosis, foaming at the mouth, up rolling of the eyes or a fixed stare, focal or generalised twitching, and jerking of the extremities. 12, 24 Atonic and tonic spells have also been reported. A postictal

period of sleepiness, or disorientation may linger for up to half an hour following the seizure. Todd's paralysis, also known as postictal palsy, could happen.[12,25]

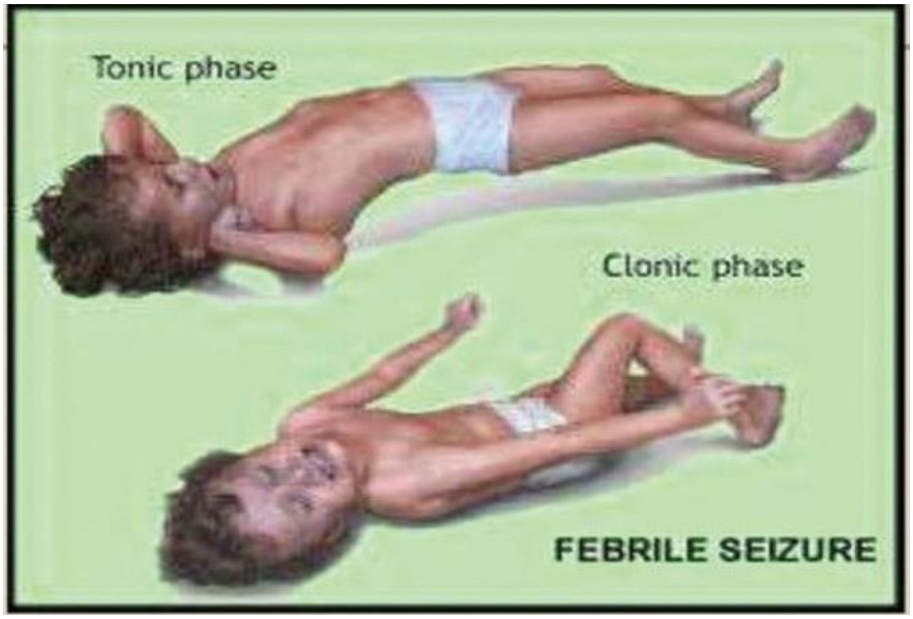


Figure 5: Tonic clonic seizures

Types of febrile seizure[23]

Simple, complicated, and febrile status epilepticus are the classifications for febrile seizures according to their length, recurrence, and presence of focal characteristics. About 5% of fever seizures are categorised as febrile status epilepticus, 25% are complex, and 70% of febrile seizures are simple. Children's status epilepticus is most frequently caused by febrile status epilepticus.

Figure 6:Difference between simple,complex and febrile status epilepticus

Simple	Complex	Febrile status epilepticus
Generalized seizures without focal features	Seizures with focal features	Seizures lasting more than 30 minutes
Less than 15 minutes duration	Seizures lasting more than 15 minutes and those lasting less than 15 minutes stopped with anticonvulsant medication	Brief serial seizures without consciousness being regained during the interictal periods with a total duration of more than 30 minutes
No recurrence within 24 hours	Recurrent seizures within 24 hours	
No preexisting neurologic abnormality	Presence of a preexisting neurologic abnormality	
	Postictal neurologic abnormality, such as Todd's paralysis	

Management[23]

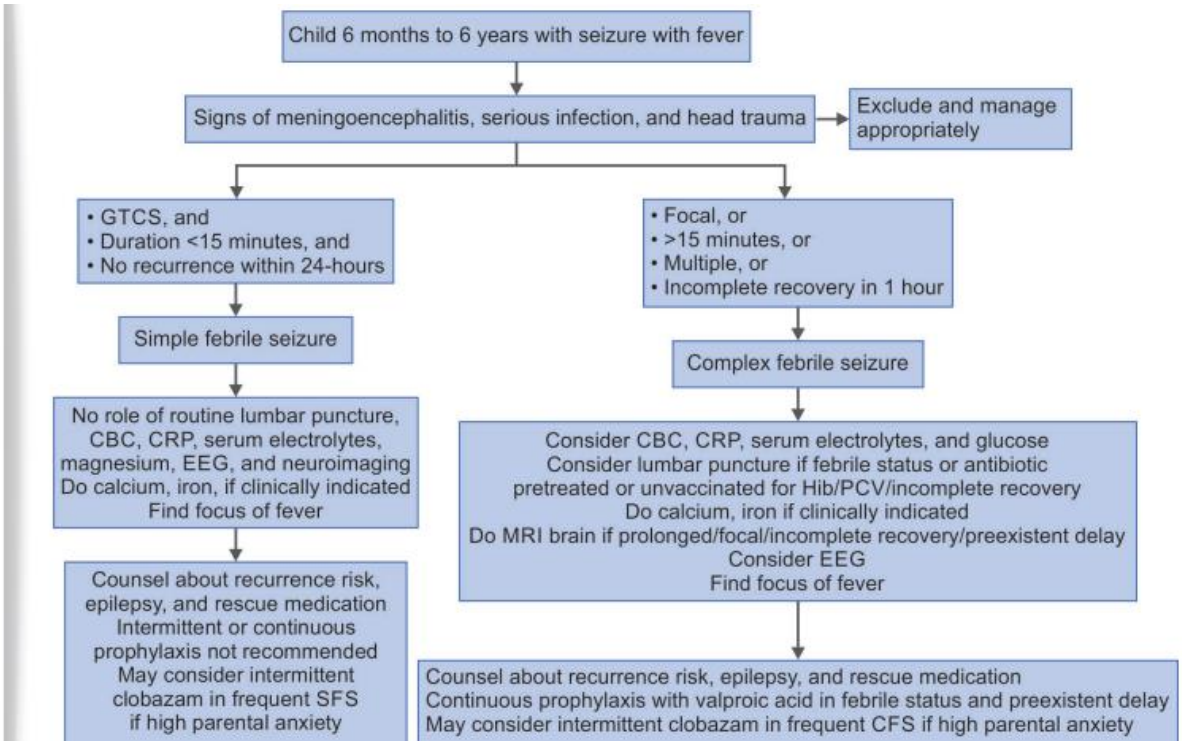
To ascertain the origin of the fever, a detail history and physical examination should be performed before evaluating a child experiencing a febrile seizure. Always important to rule out meningitis during a physical examination.

1.Laboratory findings: It is important to measure the serum glucose level of each patient who presents with seizures. Individualized laboratory testing should be performed based on the results of their physical examination and history.

2.Neuroimaging

Patients with uncomplicated febrile seizures need not undergo routine neuroimaging, according to the American Academy of Paediatrics (AAP)[26]. On an emergency basis, MRI is not necessary for the assessment of febrile status epilepticus. Advised to do neuroimaging on any patient experiencing febrile seizures having a postictal neurologic deficit, even if there are no published recommendations.

3.Lumbar puncture: A lumbar puncture is not advised as a regular procedure for all children experiencing a minor febrile seizure, according to the most recent AAP guidelines. Compared to children with simple and complex febrile seizures, children with febrile status epilepticus are more likely to develop meningitis. Lumbar puncture is advised for children aged 6 to 12 months whose immunization status is unclear. Studies have shown that 12% and 17% of children with febrile status epilepticus have bacterial meningitis.[27]



Figure[28] 7: Guidelines for febrile seizures

Treatment

Along with supportive care and assessment for potential causes of fever, there is no particular treatment recommended for simple or complex febrile seizures. There is no evidence that antipyretics can stop febrile seizures from happening again.

Less than 10% of children experience febrile status epilepticus during their first febrile seizure, even though the majority of febrile seizures are isolated events that end on their own. Intravenous benzodiazepines (such as lorazepam), rectal diazepam, or intranasal midazolam can be utilized in individuals with febrile status epilepticus or seizures that persist longer than five minutes..

GENETIC INVOLEMENT IN FEBRILE SEIZURES

Febrile seizures are seizure events that affect children during childhood febrile illness while showing no evidence of brain infection or defined medical conditions. Febrile seizures have complicated genetic patterns that involve numerous genes combined with multiple loci which frequently follow an autosomal dominant transmission with reduced penetrance. Multiple studies demonstrate that genes play a substantial role in establishing sensitivity to seizures of this nature. Six FS susceptibility regions called FEB1 through FEB6 have been found on chromosomes 8q13–q21 (FEB1) and 19p (FEB2) as well as 2q23–q24 (FEB3) and 5q14–q15 (FEB4) and 6q22–q24 (FEB5) and 18p11 (FEB6). Scientists have identified gene mutations in three sodium channel genes (SCN1A, SCN2A and A SCN1B) as well as the GABA receptor γ-2 subunit gene (GABRG2) which play a role in cases of "generalized epilepsy with febrile

seizure plus (GEFS+)" among families. Most cases of FSs together with GEFS+ lack genetic identification of their causative genes.[31]

1.GABRA GENES

The GABRA1 gene produces genetic instructions for the alpha-1 subunit that functions as part of gamma-aminobutyric acid (GABA) type A receptor required for brain inhibitory neurotransmission. Genetic alterations in GABRA1 affect the occurrence of epilepsy together with febrile seizures (FS) that normally start after body temperature increases in children.

Role of GABRA1 in Febrile Seizures

Febrile seizures affect children who are aged from 6 months to 5 years and they develop most often when body temperature increases quickly because of infections. Most febrile seizures are but some children experience unusual features when their febrile seizures occur or they may develop epilepsy later. Genetic make-up is responsible for development of febrile seizures.

Research demonstrates that GABRA1 gene defects lead to febrile seizure development in families with this condition. The GABA-A receptor dysfunction caused by these mutations creates brain signalling imbalances that reduce seizure thresholds when a child has a fever.

Mechanism

The GABA-A receptor works as an ion channel through which GABA activation brings chloride ions inside the neuron to create hyperpolarization that blocks neuronal firing potentials. Defects within the GABRA1 gene create misformed receptor components that block the receptor from performing inhibitory neurotransmission. The receptor dysfunction leads to excitability in neurons which raises the potential for seizures in high body temperatures.[32,33]

Genetic Studies and Evidence

Researchers have conducted numerous investigations which showcase the crucial place of GABRA1 in heritable reactions toward febrile seizures. **Heron et al**. [34](2007) discovered a GABRA1 gene mutation that passes through family members with febrile seizures thus strengthening the connection between this gene and FS risk. The research published by **Baulac s et al.** [35](2001) showed that GABRA1 gene mutations led to both familial febrile seizures and various epilepsy types thus proving its role in epilepsy development.

Clinical Implications

A clinical evaluation has emerged after scientists uncovered GABRA1 mutations responsible for the genetic expression of febrile seizures with associated epilepsy syndromes. Individuals born with GABRA1 gene mutations tend to develop epilepsy during their adult years although their risk grows substantially following complex febrile seizure development.

2.KCNC GENES

The KCNC1 gene produces the Kv3.1 potassium channel as an encoded product that mainly exists within neurons. Pennant channels participate in the membrane potential repolarization process after an action potential for control of neuronal firing rates and excitability. Researchers have established that KCNC1 gene mutations lead to different types of epilepsy manifestations which includes febrile seizures (FS).

Role of KCNC1 in Febrile Seizures

A febrile seizure represents a seizure condition which develops from elevated body temperature and impacts mainly young children exhibiting brief and brief seizures. The KCNC1 genetic mutations along with other variants enhance febrile seizure risk which may result in subsequent

epilepsy development. The Kv3.1 channel helps neurons to rapidly repolarize especially in high-frequency neuron regions of the hippocampus and cortex that control seizure generation. Brain channel defects cause abnormal nerve discharge patterns which increases the risk of seizures especially when patients experience fever.

Mechanism of Action

The Kv3.1 channel enables neurons to discharge at high frequencies without property becoming excessively excited. The normal function of KCNC1 channels becomes impaired by gene mutations which leads to changes in cell depolarization characteristics and elevated neuronal activation levels. When neurons suffer from this dysfunction they tend to fire uncontrollably especially in reaction to fever triggers thus creating seizure vulnerability.

A change in body temperature leading to febrile seizures will exacerbate neuronal excitability particularly when the individual carries KCNC1 mutations. The main effect of poor Kv3.1 channel performance becomes the inability to restore proper membrane potential after an action potential which results in prolonged neuronal firing that causes seizures.

Genetic Studies and Evidence

Multiple research has discovered that febrile seizures and other seizure disorders exist due to KCNC1 gene mutations. A research by **Weckhuysen et al.** [36](2012) demonstrated that KCNC1 gene mutations cause epileptic encephalopathy even though initial symptoms did not include febrile seizures yet patients sometimes experienced such seizures early in their epilepsy development. Scientists believe KCNC1 represents a genetic substance that raises the risk of febrile seizures when people interact with temperature-related events.

Muona et al.[37] (2016) reported structural deviations in KCNC1 genes to result in episodic ataxia alongside seizures whereas specific patients showed febrile seizures as an accompanying

symptom. Scientific data shows that faulty KCNC1 genetic code causes dysfunction of potassium channels which creates both movement disturbances and increased seizure risks.[38]

Clinical Implications

The clinical comprehension of KCNC1's function in febrile seizures maintains significant practical value. The detection of KCNC1 mutations at an early stage enables healthcare providers to assess patient vulnerability to febrile seizures and design strategies to oversee individuals whose family lineage includes febrile seizure and epilepsy. Studies demonstrate that KCNC1 mutations serve to explain how certain epileptic syndromes evolve when febrile seizures transform into more serious forms of epilepsy.

3.SCN1A GENES

The SCN1A gene directs the production of Nav1.1 which represents the voltage-gated sodium channel alpha subunit that controls sodium entry through neurons for action potential conduction. These channels appear at high levels in brain cells that produce GABAergic inhibition particularly in sections such as hippocampus and cortex regions. Scn1a gene variants exist as a leading risk factor for febrile seizure (FS) development particularly when a person inherits genetic components that make them more vulnerable to these disorders.

Role of SCN1A in Febrile Seizures

When children develop sudden high temperature spikes because of infections a brain seizure may occur. When a person has these episodes they usually stay benign yet the chance of developing extreme forms of epilepsy increases when a person has genetic susceptibility. Science has established a direct association between the SCN1A gene and febrile seizures along

with other conditions including epilepsy or FS+ which show increased risk for future epilepsy development.[39]

Mechanism of Action

Measurable action potentials that spread within neurons depend on the Nav1.1 channel which gets its instructions from the SCN1A gene. Scn1a gene mutations create defects that break down sodium channel abilities therefore decreasing brain inhibitory operations which results in hyperexcitable neurons. Neuronal hyperexcitability becomes more likely when inhibitory control decreases because molecular changes increase electrical sensitiveness especially during fever which boosts neuronal firing rates.[40]

The body becomes more active at neuronal levels as body temperature rises from fever onset during typical situations. The efficient operation of Nav1.1 sodium channels depends on unaffected SCN1A genes because gene mutations lead to inefficient sodium channel function that causes excessive neuronal firing and reduced seizure thresholds. Seizure risk increases in people with enhanced brain excitability which can progress to severe disorders of epilepsy including Dravet syndrome in some cases.

Researchers from Zuberi et al.[41] (2006) discovered that SCN1A mutations appear frequently in patients who have febrile seizures together with early-onset epileptic encephalopathy. The discovery indicates that SCN1A gene mutations create higher risk for condition vulnerability to febrile seizures while also increasing seizure disorder severity.

Clinical Implications

Diagnosing and treating patients becomes more effective due to SCN1A mutation identification practices. Early SCN1A mutation tests enable healthcare providers to identify children who are more likely to develop febrile seizures particularly when they share familial links with individuals who have febrile seizures plus or epilepsy. When SCN1A mutations become

detected healthcare providers can modify treatment plans while establishing increased patient observation to stop potential complications from happening.

The SCN1A mutation status helps determine future risk for Dravet syndrome evolution along with other epilepsy types after febrile seizures therefore guiding antiepileptic drug (AED) selection that impacts sodium channel effects and inhibitory neurotransmission strength.

4.ALDH7A GENES

Through expression of the ALDH7A1 gene, the body produces aldehyde dehydrogenase 7A1 enzyme that aids in processing lysine and eliminating body-detoxifying aldehydes. The enzyme exists mainly in liver tissues, kidneys and brain tissues. Scientific studies confirm that ALDH7A1 gene abnormalities result in antiquitin deficiency, leading to the development of pyridoxine-dependent epilepsy during infantile periods. This severe epilepsy represents the worst form of the condition. The dysfunction of the ALDH7A1 gene increases the risk of seizures, which include febrile seizures within specific genetic settings, despite not causing common febrile seizures directly.

Role of ALDH7A1 in Febrile Seizures

Epilepsy due to pyridoxine dependence develops from inherited ALDH7A1 gene defects which reduce the activity of the ALDH7A1 enzyme. The enzyme deficiency causes toxic byproduct α-AASA from lysine metabolism to build up which disrupts neurotransmission and raises seizure risk specifically for febrile seizures.

Children born with ALDH7A1 genetic anomalies develop seizures that mainly manifest as febrile seizures soon after birth and resist typical anticonvulsant drugs. The seizures of PDE respond to vitamin B6 treatment also known as pyridoxine because this supplement bypasses metabolic defects to minimize neurotoxic aldehyde formation.[42]

Mechanism of Action

The metabolism of lysine depends significantly on the activity of ALDH7A1 which transforms the compound α-aminoadipic semialdehyde into α-aminoadipate. The changes in ALDH7A1 activity lead to build-up of α-AASA which produces interference in GABAergic neurotransmission. The brain mainly relies on GABA (gamma-aminobutyric acid) as its primary inhibitory neurotransmitter and its dysfunctional regulation results in epilepsy. The built-up α-AASA creates a decline in pyridoxal phosphate (PLP) which serves as the active vitamin B6 form necessary for GABA production. An impaired GABAergic system causes heightened neuronal response and thus makes individuals more vulnerable to seizures which includes febrile seizures.[43]

When symptoms of fever exist the brains of children carrying ALDH7A1 mutations face elevated seizure risk because their GABA inhibitory controls remain reduced. Studies imply that mutations in ALDH7A1 create increased susceptibility to febrile seizures especially among people whose genes already predispose them toward these seizures.

Genetic Studies and Evidence

Multiple research papers have validated how ALDH7A1 mutations cause pyridoxine-dependent epilepsy so that the condition frequently includes febrile seizures as one of its chief manifestations. Lab analyses by **Gunter et al.**[44] (2009) determined that ALDH7A1 mutations exist within the DNA of patients suffering from pyridoxine-dependent epilepsy and create α-AASA buildup which damages GABAergic signaling functions. The authors pointed out febrile seizures as an initial presentation of this disorder.

Karnebbek et al[45] reported in their study that pyridoxine-dependent epilepsy manifests several neurological and systemic symptoms within its broad phenotypic range. The disease is known

for producing seizures that remain uncontrolled among infants during their first year. Early diagnosis of pyridoxine-dependent epilepsy requires immediate attention since its successful management involves both lowering lysine levels through specific approaches and using pyridoxine supplementation to achieve improved seizure control and improved developmental results. Medical professionals should conduct α-aminoadipic semialdehyde/pyrroline 6' carboxylate and ALDH7A1 molecular analysis for antiquitin deficiency to identify the cause of unexplained seizures in every affected infant.

MATERIALS AND METHODS

Source of Data

All cases of seizures with fever admitted to the Paediatrics Department at Shri B.M. Patil Medical College Hospital and Research Centre, Vijaypura, that meet the inclusion and exclusion criteria will be included in the study. A minimum of 50 cases were studied.

Method of Data Collection

Children who meet the selection criteria will be included after obtaining written informed consent from their parents.

Selection criteria

Inclusion criteria:

An event in infancy or childhood usually occurs between 6 months and 6 years of age associated with fever but without evidence of intracranial infection.

All children who fulfill the definition/ criteria of febrile seizures.

Exclusion criteria:

Children with gross neurological deficits

Children with atypical seizures

METHOD OF STUDY

Clinical Sample (Blood) Collection:

After being enrolled in the study, the subjects gave their consent. One millilitre of peripheral blood was drawn and stored at 4°C in EDTA-coated vacutainers (BD367863) after consent was obtained.

Isolation of Genomic DNA and Quantification

From 300µl of peripheral blood genomic DNA was isolated, with the help of a commercial DNA isolation kit (Bangalore Genei, India).

Genomic DNA Isolation Protocol:

1. In a 1.5 ml EDTA-coated vial 300 µl of peripheral blood was collected.

2. By adding1 ml of 1 X solution A (provided by the kit) RBC cells were lysed.

3. At room temperature the vials were centrifuged for 5 min at 8000 RPM.

4. Until a clear white WBC pellet was obtained the above step was repeated.

5. 600µl of solution B was added (provided by the kit) to the WBC and mixed gently for clear lysis.

6. It was centrifuged at room temperature for 10 min at 10,000 RPM.

7. The Supernatant was collected and 0.9 ml absolute cold ethanol was added to it and mixed.

8. Centrifuged at 4oCfor 20 min, at 10,000 RPM.

9. Precipitate DNA was washed with 0.5 ml of 75% ethanol.

10. Centrifuged for 5 min at 10,000 RPM.

11. 100 µl of solution C was added (provided by the kit) after air drying the DNA pellet.

12. The vial was incubated at 55oC for 10 min.

13. To remove any insoluble materials it was centrifuged at 10,000 RPM for 2 min.

14. The DNA thus obtained was stored at -20oC until further use.

The quality of the isolated DNA was checked under gel electrophoresis. 100 ml of 1% agarose gel was prepared (1 gm of Agarose + 100 ml of 1X TAE buffer). The same isolated DNA was quantified under "Nanodrop" (Quawell) and the quantity and quality of the DNA were reported.

Exon-specific intronic primer designing:

The web-based freely available program "Primer3" which is widely accepted was used, (http://frodo.wi.mit.edu/ primer3/ input. Html) for designing PCR primers. Primer 3 is a Bioinformatics tool that helps in designing the primers for the target region in the given nucleotide sequence as per the requirement of the user or applications. The designed primers using Primer 3 were reconfirmed for the specificity of its binding site using the web-based bioinformatics tool "Genome Build 36" (https://genome.ucsc.edu/FAQ/FAQreleases.html), and for its Insilco amplification on "Insilco PCR" (http://insilico.ehu.es/PCR/). All the designed primers for our target genes or region are tabulated in table No. 1 along with the annealing

temperature and amplicon size. Primers were got synthesized by a commercial oligo synthesizer (MWG Biotech, India).

Details of the primer sequences and annealing temperatures used for the amplification of.

Sl.No	Primer ID	Sequence	Product Size	Annealing Temperature
1				
2				
3				
4				

Polymerase Chain Reaction (PCR):

PCR reactions contained 0.5 µl of genomic DNA (75ng/µl to 150 ng/µl at a concentration of 75 ng/µl) together with 0.5µl of each primer (5pmol), 0.4µl of dNTP (10pmol), 0.2µl Taq DNA polymerases (3units/ µl) beside 4 µlTaq Buffer (5X) (BioRad, USA) before the total solution quantity reached 20µl using molecular biology grade water. Master cycler gradient (Eppendorf, Germany) operated under the conditions of 980C denaturation for 10sec followed by 35 cycles at 980C for 10sec (cycle denaturation) and 720C extension for 15sec. The annealing temperature of the primer determined the primer incubation temperature (Table-1) as 720C for 15 seconds followed by primer extension at 720C for 5 min. PCR products underwent 100bp standard size verification via gel electrophoresis.

PCR required initial denaturation at 98°C for 10 seconds followed by denaturation at 98°C for 10 seconds then annealing depending on the primer for 10 seconds at 72°C for 5 minutes and finished with a 4°C hold.

Agarose Gel Electrophoresis

Gel electrophoresis functions as a molecular biology method which splits DNA and RNA according to their fragment length. This widely accepted techniques provides an estimate of DNA and RNA fragment sizes and separates proteins based on their charges. The electric field separates negative nucleic acid molecules which travel through an agarose matrix. The mobility of shorter molecules surpasses that of longer ones since shorter molecules penetrate the gel pores effortlessly. This phenomenon is called sieving.

DNA Sequencing (Capillary Based)

PCR products were subjected for capillary based Big-Dye terminator sequencing. Prior to sequencing, the PCR products were subjected to cycle sequencing and plate processing.

Cycle Sequencing

The cycle sequencing method was used to perform Big-Dye labelling and chain termination in accordance with the Sanger Sequencing procedure. The PCR amplicon was put through a cycle sequencing procedure using a single primer to mark each base. Cycle sequencing was done using the Big-Dye TM terminator v3.1 (Applied Biosystems, USA) in accordance with

the manufacturer's instructions. By the primers' annealing temperature, cycle sequencing of the PCR products was performed.

Cycle sequencing PCR mixture constituents

SL. No.	Constituents	Quantity
1	Molecular Biology grade water	6.3 µL
2	Big Dye Buffer (5X)	1.3 µL
3	Big Dye	1.0 µL
4	Template (PCR product)	1.0 µL
5	Forward Primer	0.2 µL
6	Reverse Primer	0.2 µL
Total		**10 µL**

Note: Only one of the primers i.e either forward or reverse primer was used during cycle sequencing

The cycle sequencing conditions

process	Temperature	Time
Initial denaturation	98	10sec
Denaturation	98	10sec
Annealing	Primer dependent	10sec
Elongation	72	5min
Hold	4	

Note: The annealing temperature is primer dependant and varies for each primer.

Sequencing Clean-up (Plate Processing)

Each sample received 50µl of 100% ethyl alcohol and 2µl of 3M sodium acetate, which precipitated the DNA for 15 minutes at room temperature in order to eliminate the unbounded fluorescent DNTPs from the terminator sequencing procedure. For 30 minutes at 4°C, the samples were centrifuged at 4000 rpm. The reaction plate was centrifuged in reverse for 20 seconds at 300 rpm after the supernatant was disposed of. Following the addition of 100µl of 75% alcohol, each sample was centrifuged for 15 minutes at 25°C at 4000 rpm. The supernatant was discarded and the plate was centrifuged in a reverse manner at 300 rpm for 20 seconds to remove the alcohol completely. The plate was dried at room temperature until the last drop of alcohol dripped off. 10µl of Hi-Di Formamide was added to each well of the sample plate. The samples were heated to 96°C for 5 minutes and immediately cooled to 4°C to denature and linearise the cycle sequencing products. The processed products were loaded in the sequencer for sequencing.

Sequencing Run

Data collecting software was used to import sample information sheets that included analysis methodologies and sample details. To produce DNA sequences or electropherograms, prepared samples were examined using an ABI 3730 genetic analyser (Applied Biosystems, USA). Following the sequencing reaction, Sequencing Analysis v5.4 software (Applied Biosystems, USA) was used to assess the quality of the produced sequence.

Sequence Alignment

Variant reporter software (ABI v1.1) was used to match the produced sequences to their corresponding reference sequences. One of Applied Biosystems' compatible programmes for automated sequencing data analysis is the variant reporter. It compares sequences for insertions, deletions, known variations, and new mutations. By contrasting the consensus sequences with a recognised reference sequence, it enables study of the resequenced data. As the software's default programme, the variant reporter's results were tabulated in PDF format.

DATA ANALYSIS:

Determination of sample size:

With an anticipated Incidence of Febrile seizures of 2-5% the study would require a sample size of 50

Patients with 95% level of confidence and 6% absolute precision,

Formula used:

 n=z 2 p*q

d 2

Where Z= Z statistic at α level of significance

d 2 = Absolute error

P= Proportion rate

q= 100-p

STATISTICAL ANALYSIS

A Microsoft Excel sheet containing the collected data will be used for statistical analysis, which will be carried out using the statistical programme for the social sciences (Verson 20). Mean ±SD, median and interquartile range, frequency, percentages, and graphs will be used to display the results.

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RESULTS:

Pilot Study

Owing to the fact that genetic analysis of febrile convulsions has limited evidence, a Whole Exome sequence of 12 samples was done as a pilot study.

All samples came negative for the above-mentioned genes, and a Whole Exome Sequencing was planned for the same 12 samples for which the pilot study was done.

Table 1: Results of the Whole Exome Sequencing.

Table 2: Analysis Results for Normal samples		
Sample ID	**Analysis Result**	**Copy Number Variation (CNV)**
1F	No significant variant has been identified	Not Detected
2F	No significant variant has been identified	Not Detected
3F	No significant variant has been identified	Not Detected
4F	No significant variant has been identified	Not Detected
5F	No significant variant has been identified	Not Detected
6F	No significant variant has been identified	Not Detected
7F	No significant variant has been identified	Not Detected
8F	No significant variant has been identified	Not Detected
9F	*CPA6* gene identified as an additional gene	Not Detected
10F	*SEMA6B* gene identified as an additional gene	Not Detected
11F	*ALDH7A1* gene identified as an primary gene	Not Detected
12F	*SAMD12* gene identified as an additional gene	Not Detected
	KCNQ2 gene identified as an primary gene	

Based on the above-mentioned results of the Whole Exome Sequencing, genes were selected, and the study was conducted.

Table 2: Age-wise distribution among study participants

Sl. no	Age	Frequency (n)	Percentages %
1	<12 months	2	4.0
2	1-2 years	25	50.0
3	2-5 years	20	40.0
4	>5 years	3	6.0
5	Total	50	100.0

Age-wise distribution of study participants found that the majority were in 1-2 years, i.e. 50% (n-25), followed by 2-5 years 40%(n-20), and the least in >12 months of age, and it is shown in the bar diagram

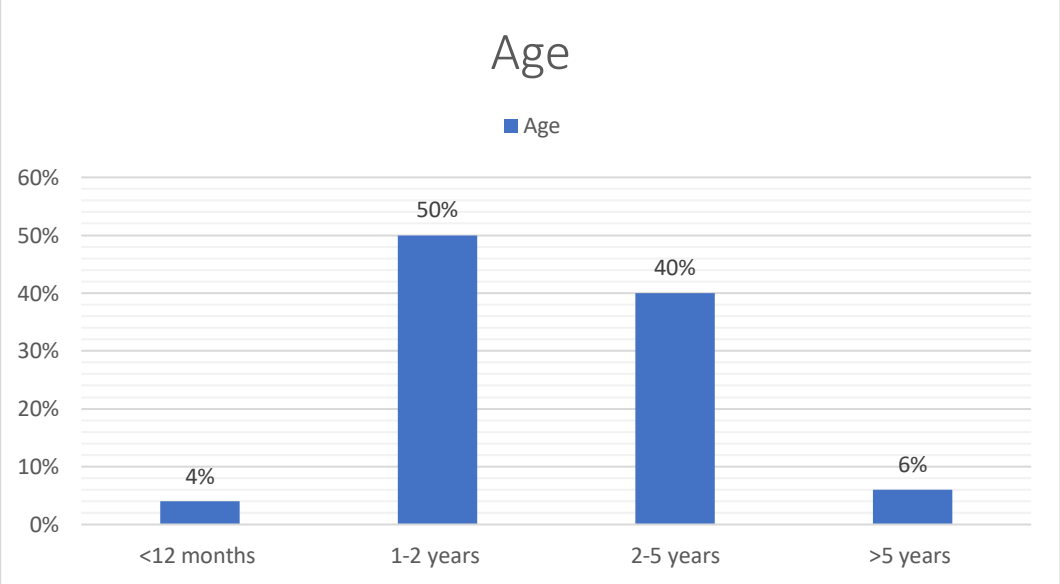


Figure 8: **Age-wise distribution among study participants**

Table 3: Gender distribution among study participants

Sl no	Sex	Frequency (n)	Percentages %
1	Females	22	44
2	Males	28	56
3	Total	50	100.0

This is the table showing the gender-wise distribution of study participants with febrile seizures. It found that 56%(n-28) were males and 44%(n-22) were females and it is shown in the pie diagram.

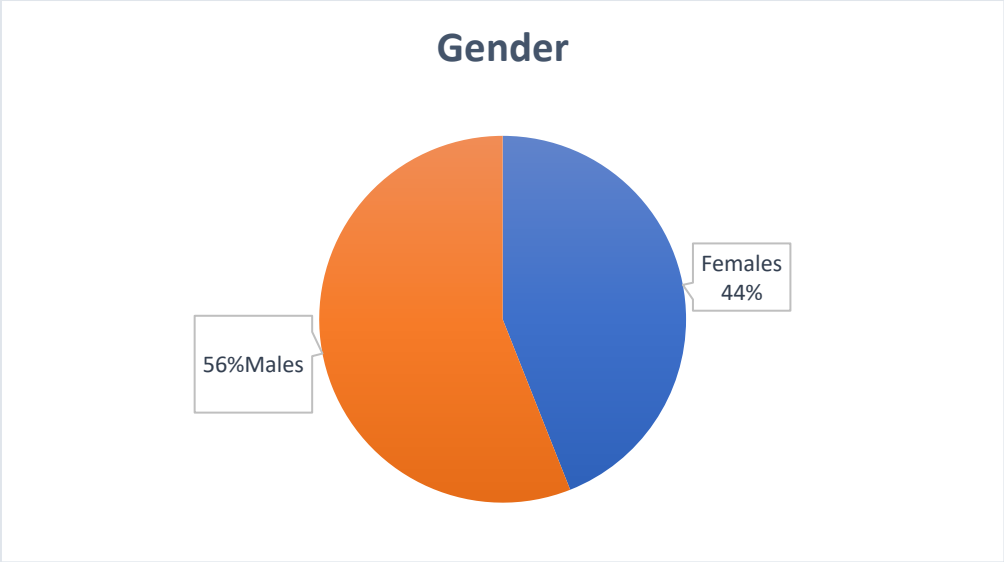


Figure 9: Gender distribution among study participants

Table 4: Distribution of Past history among study participants

Sl no	Past history	Frequency (n)	Percentages %
1	No	30	60
2	Yes	20	40
3	Total	50	100.0

This is the table showing the Past history distribution of study participants with febrile seizures and found that 40% (n-20) had a past history of febrile seizures, and 60% (n-30) had no past history, and the same is shown in the pie diagram.

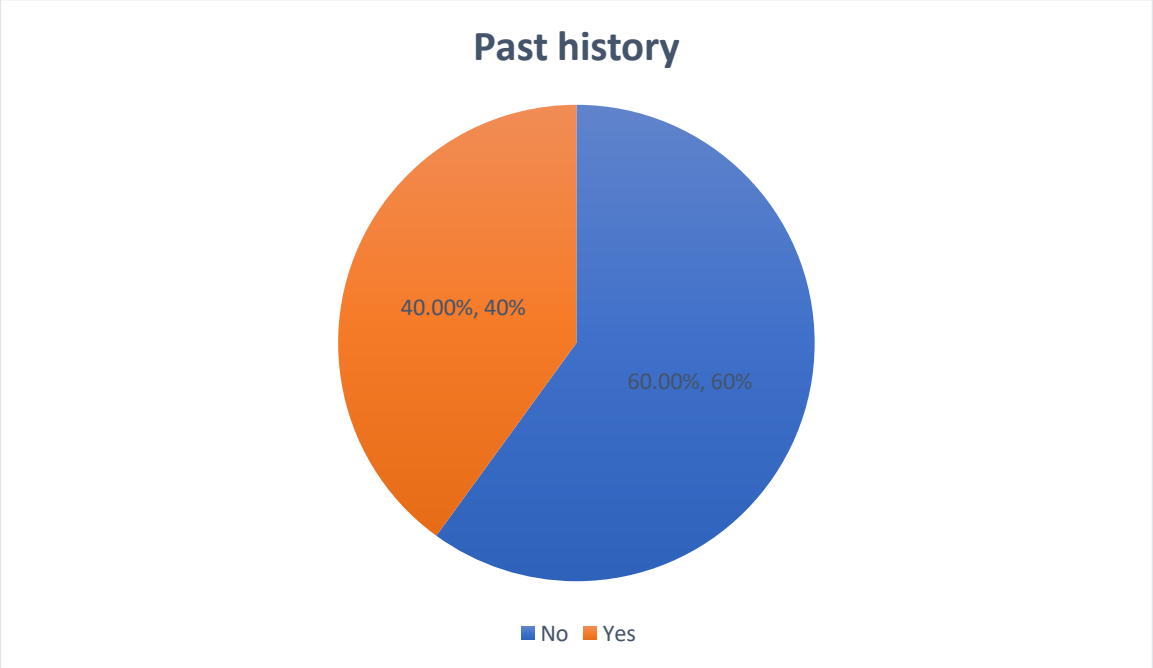


Figure 10: Distribution of Past history among study participants

Table 4: Distribution of family history of febrile Convulsion among study participants

Sl no	Family history of febrile Convulsion	Frequency (n)	Percentages %
1	No	31	62
2	Yes	19	38
3	Total	50	100.0

This is the table showing the family history of Febrile Convulsions and the distribution of study participants with febrile seizures. It was found that 38% (n-19) had a family history of febrile convulsions, and 62%(n-31) had no family history of febrile convulsions and it is shown in the bar diagram.

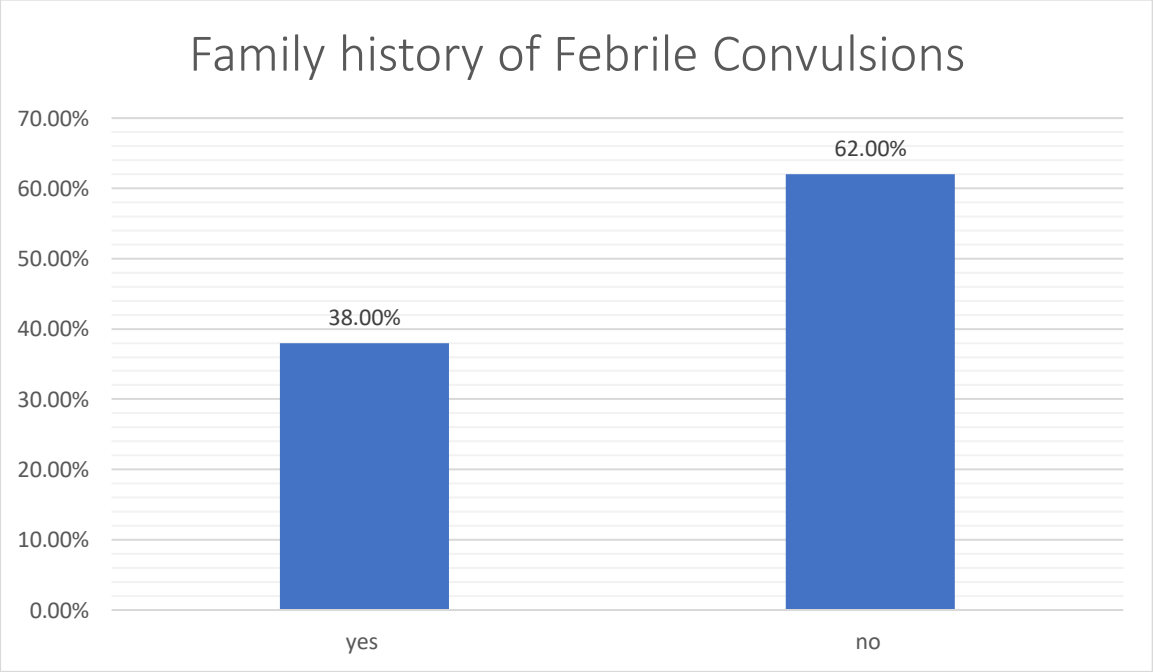


Figure 11: Distribution of family history of febrile Convulsion among study participants

Table 6: Distribution of Degree of relation among study participants

Sl no	Degree of relation	Frequency (n)	Percentages %
1	First degree	10	20
2	Second Degree	8	16
3	Third Degree	2	4
4	No Relation	30	60
5	Total	50	100

This is the table showing the Degree of relation with family-distribution of study participants with febrile seizure and found that 20% (n-10) had First degree relative with a history of febrile convulsions followed by 16% (n-8) had Second degree relative and then followed by 4% (n-2) had third degree relative and it is shown in bar diagram.

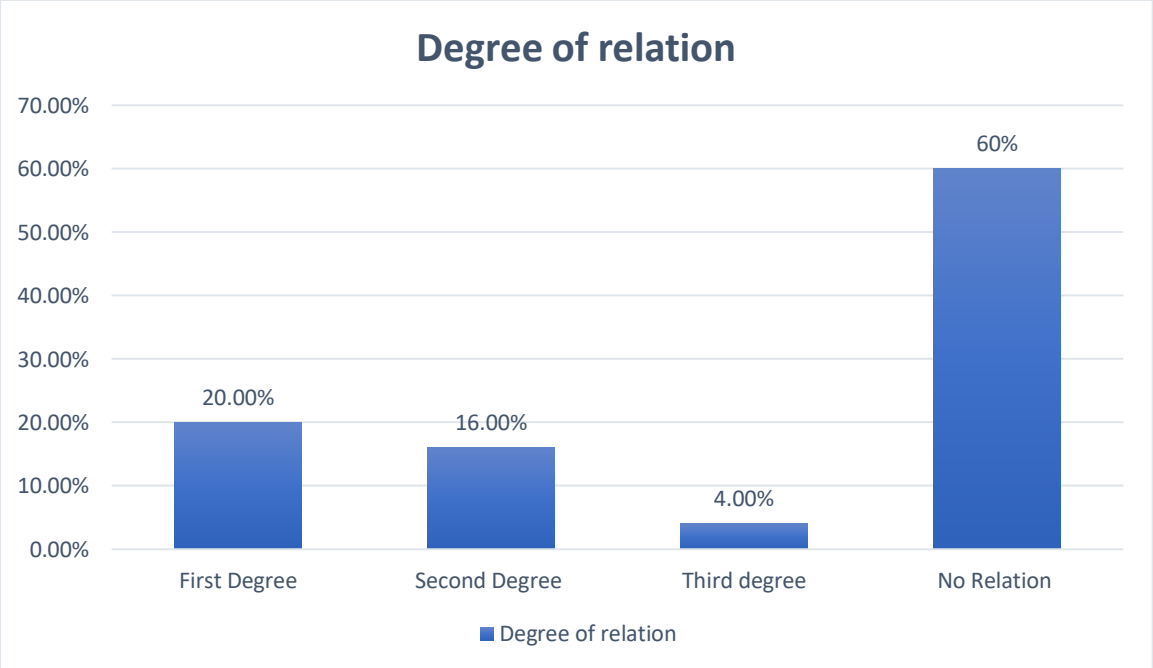


Figure 12: Distribution of Degree of relation among study participants

Table 7: Distribution of Birth order among study participants

Sl no	Birth order	Frequency (n)	Percentages %
1	1	25	50
2	2	24	48
3	3	1	2
4	Total	50	100.0

This is the table showing the Birth order- distribution of study participants with febrile seizures. It was found that among the study participants, 50%(n-25) had a birth order 1 followed by 48% had a birth order 2, and the least 2%(n-1) had a birth order 3. It is shown in the bar diagram.

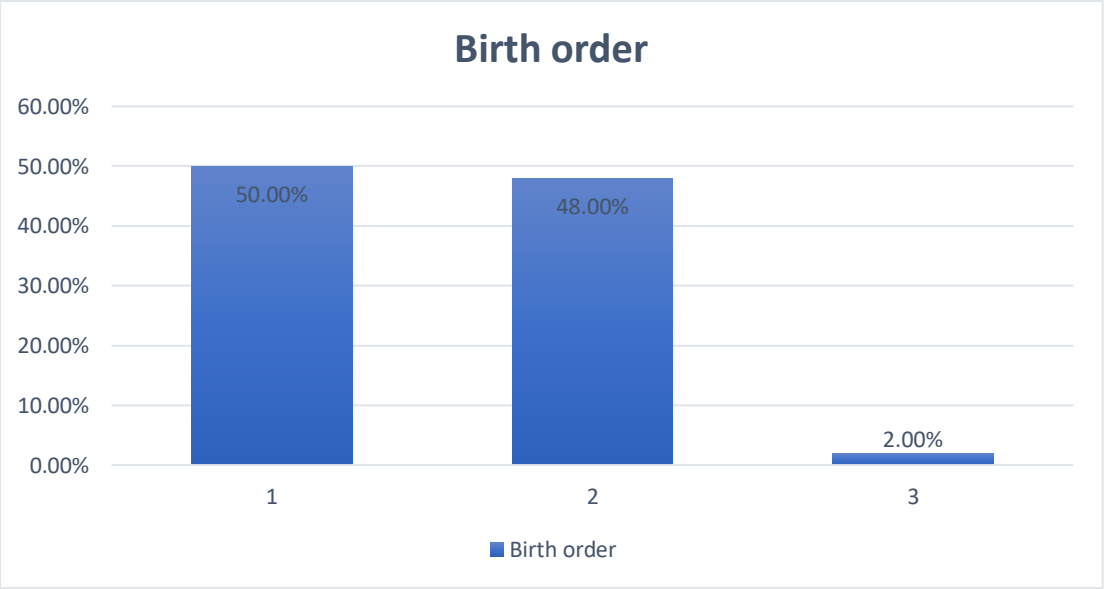


Figure 13: Distribution of Birth order among study participants

Table 8: Distribution of On Tab Clobazam prophylaxis during fever episodes among study participants

Sl no	On Tab Clobazam during fever episodes	Frequency (n)	Percentages %
1	No	30	60
2	Yes	20	40
3	Total	50	100.0

This is the table showing the **On Tab Clobazam prophylaxis during fever episodes** distribution of study participants with febrile seizure, and it was found that among the study participants, about 40% (n-20) were taking Clobazam tab prophylaxis during fever episodes, as shown in the pie diagram.

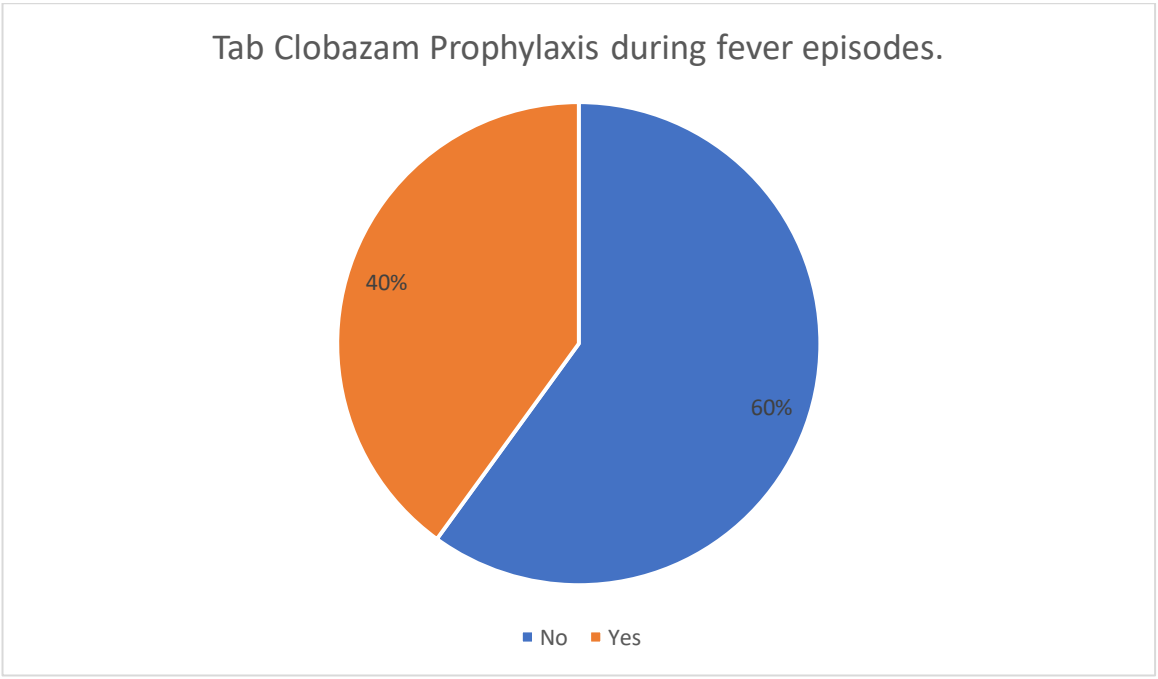


Figure 14: Distribution of On Tab Clobazam during fever episodes among study participants

Table 9: Distribution of Type of febrile convulsion among study participants

Sl no	Type of febrile convulsion	Frequency (n)	Percentages %
1	Atypical Febrile Convulsions	12	24
2	Typical Febrile Convulsions	38	76
3	Total	50	100.0

This is the table showing the **Type of Febrile seizures**; distribution of study participants with febrile seizures was found that the majority had typical febrile seizures, 76% (n-38) and 24% (n-12) had atypical febrile seizures and shown in the bar diagram.

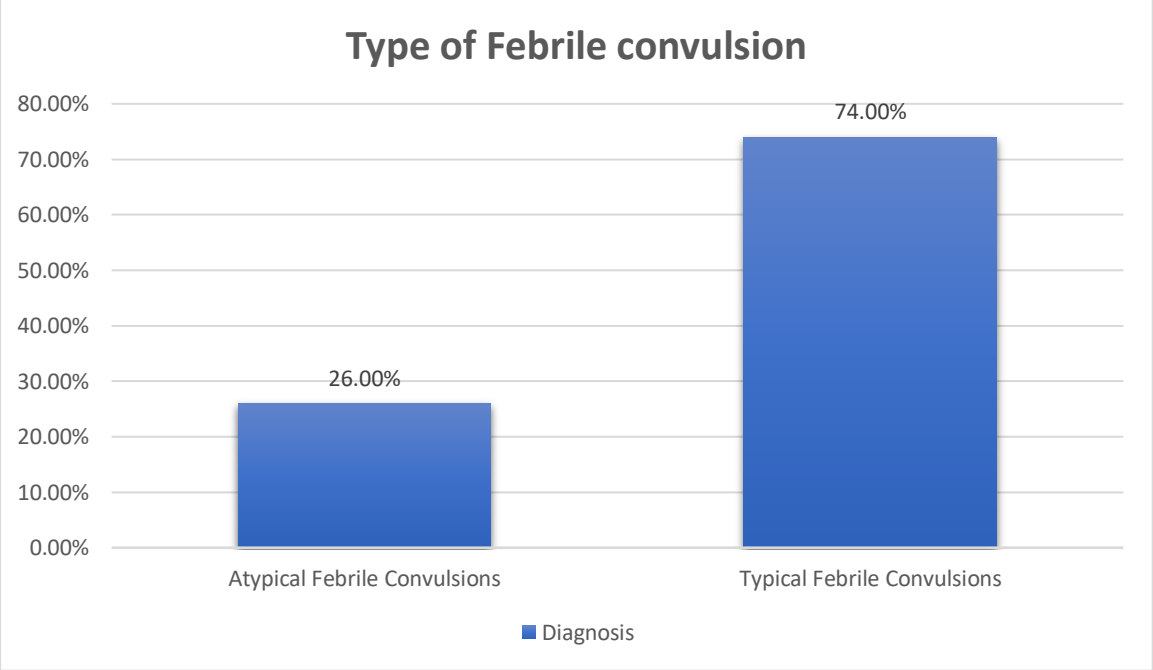


Figure15: Distribution of Type of Febrile convulsions among study participants

Table 10: Distribution of Haemoglobin level among study participants

Sl no	Hb LEVEL	Frequency (n)	Percentages %
1	<11	33	66
2	>11	17	34
3	Total	50	100.0

This table represents the Hb level, 66% (n-33) had Hb level <11mg/dl and 34% (n-17) had Hb level >11mg/dl. The mean Hb level is 9.92 mg/dl and is shown in the bar diagram.

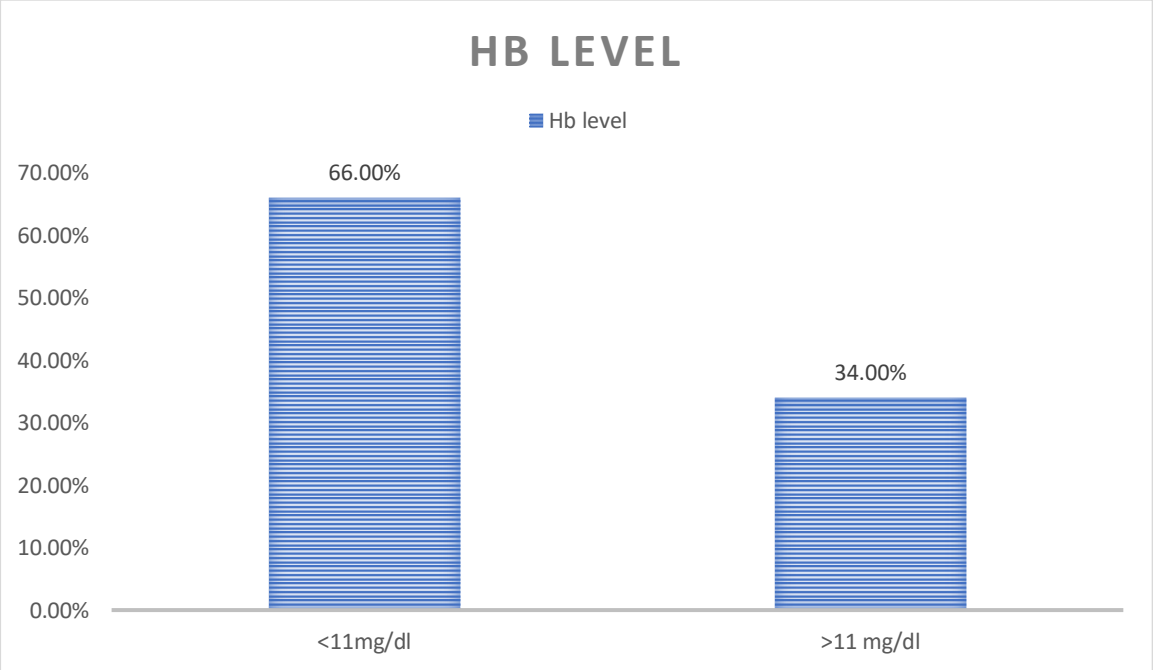


Figure 16: Distribution of Haemoglobin level among study participants

Table 11: Distribution of Lumbar puncture among study participants

Sl no	Lumbar puncture	Frequency (n)	Percentages %
1	Done	28	56
2	Not done	22	44
3	Total	50	100

This table represents the Lumbar puncture done for study participants and found that 28(56%) had undergone lumbar puncture, and 22(44%) had not undergone a lumbar puncture it is shown in the pie diagram.

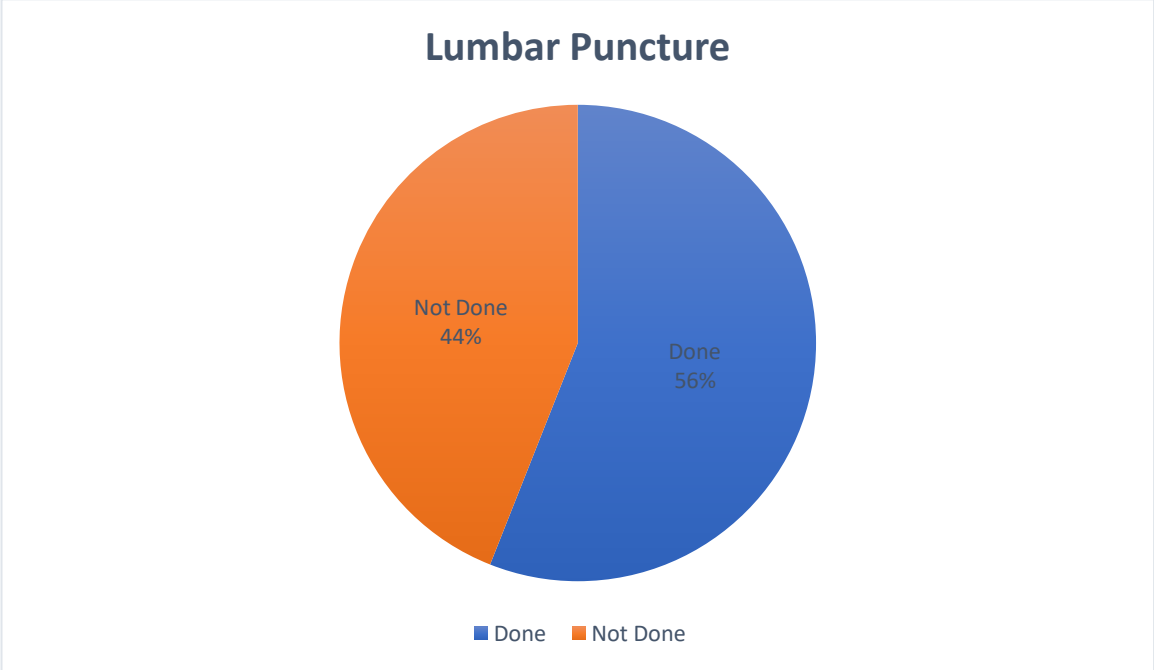


Figure 17: Distribution of Lumbar puncture among study participant

Table 12: Association between Family history and type of febrile seizure

Sl no	Family History of Febrile convulsions	Atypical Febrile Convulsions	Typical Febrile Convulsions	P value
1	No	6(50%)	25(65.8%)	0.384
2	Yes	6(50%)	13(34.2%)	
3	Total	12(100%)	38(100%)	

χ 2=9.65,df-1

The table shows the association between family history of febrile convulsions and the type of febrile convulsion (atypical vs. typical) among the study participants. Among children with no family history of febrile convulsions, 50% (n=6) experienced atypical febrile convulsions, while 65.8% (n=25) had typical febrile convulsions. In contrast, children with a positive family history of febrile convulsions showed a higher prevalence of atypical febrile convulsions (50%, n=6), whereas 34.2% (n=13) had typical febrile convulsions. The obtained p-value (0.384) suggests no significant association between a family history of febrile convulsions and the type of febrile convulsions..

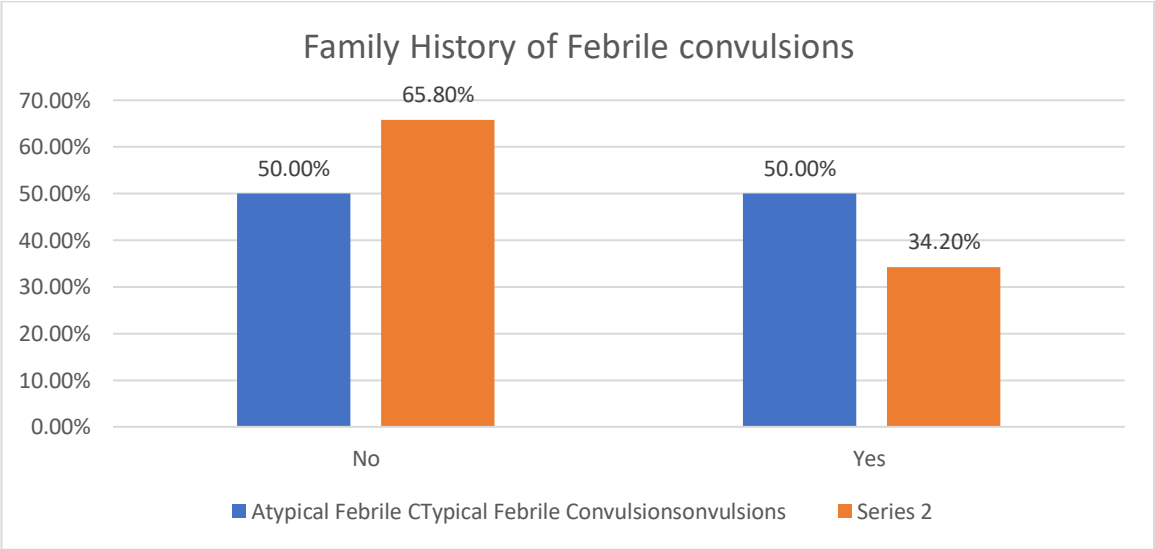


Figure 18: Association between Family history and type of febrile seizure

Table 13: Association between past history and type of febrile seizure

Sl no	Past history	Atypical Febrile Convulsions	Typical Febrile Convulsions	P value
1	Yes	7(58.3%)	13(34.2%)	0.304
2	No	5(41.7%)	25(64.5%)	
3	Total	12(100%)	38(100%)	

χ 2=0.450,df-1

The table shows the association between past medical history and the type of febrile convulsions. Children with a positive past history had a higher prevalence of atypical febrile convulsions 17 (58.3%) compared to typical febrile convulsions 13 (34.2%), whereas those with no past history predominantly experienced typical febrile convulsions (64.5%). The p-value(0.304) indicates no significant association,

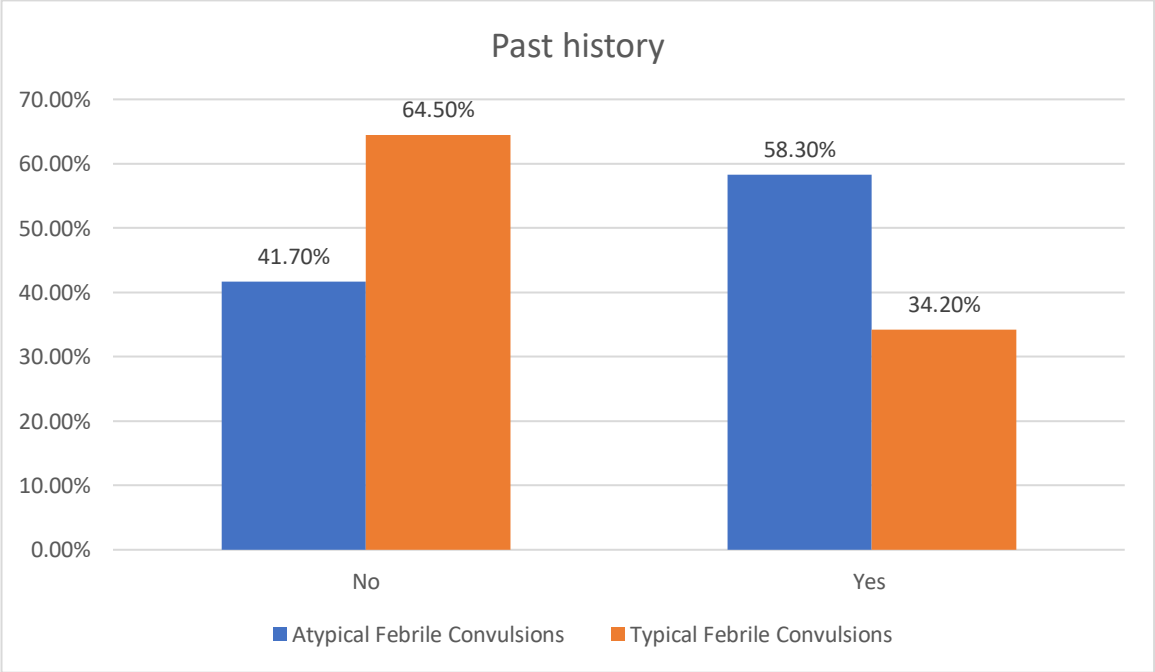
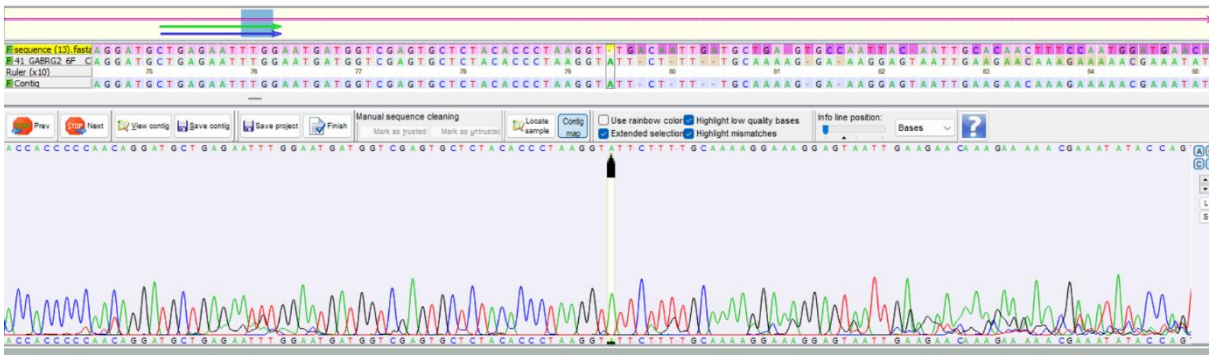
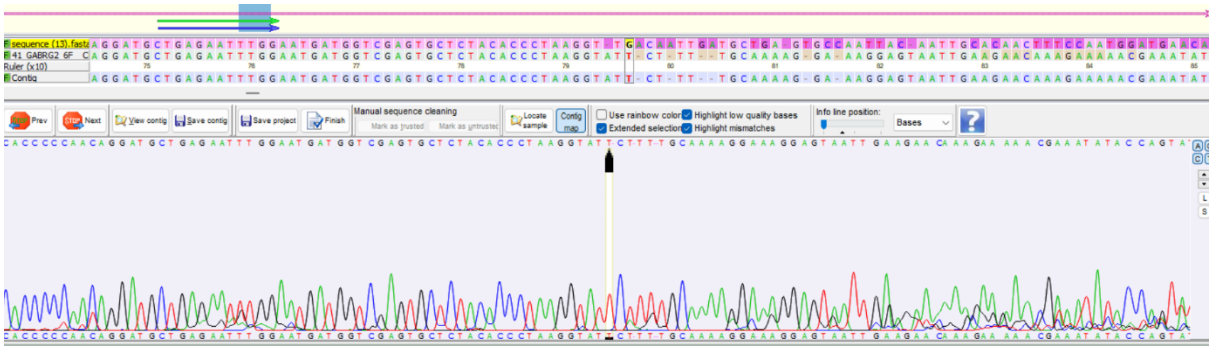
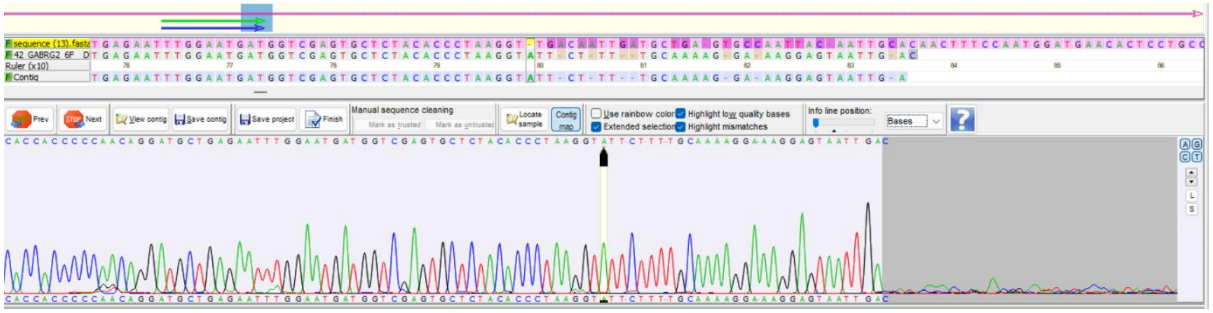


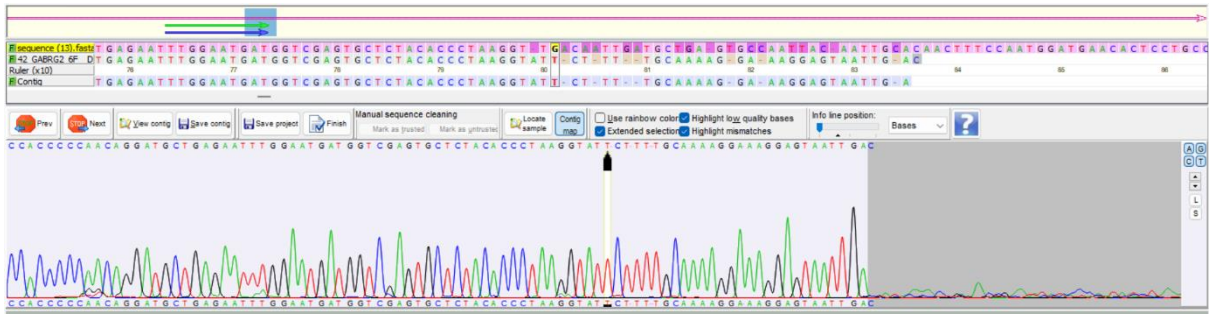
Figure 19: Association between Past history and Type of Febrile seizures.

GABRG2 MUTATION ANALYSIS







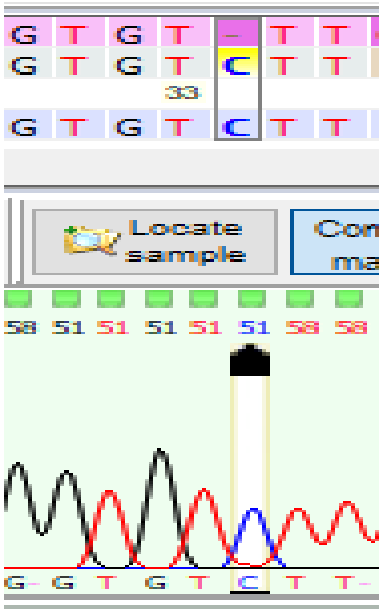


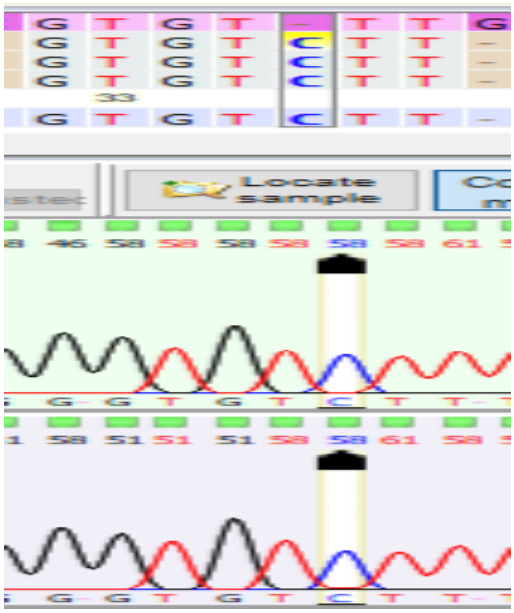
Summary:

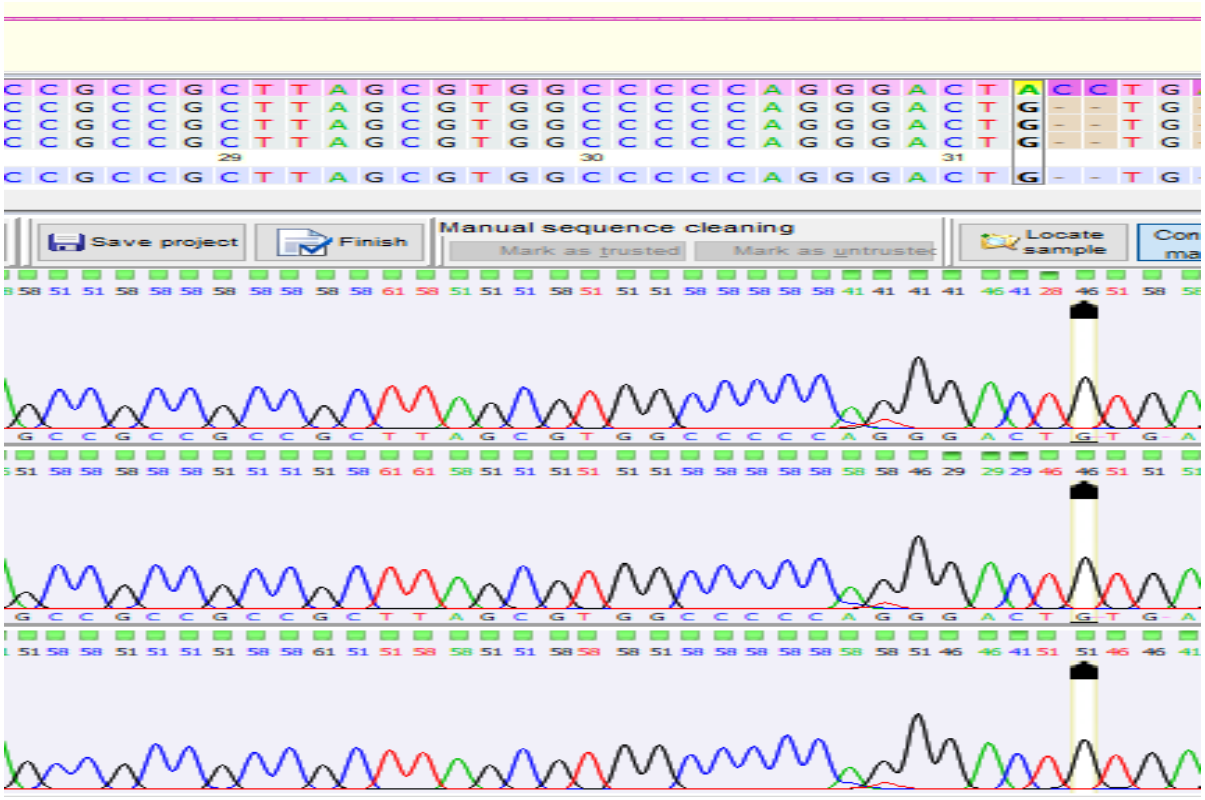
Two mutations were identified in 2 in all the samples. However, these variants do not match any recorded entries in the Ensembl database [Ensembl genome browser 113] Due to the presence of noise in the nearby sequence, it is currently uncertain whether these are true mutations. To establish these as novel variants, sequence more samples to confirm the consistency and validity of the variants and perform functional validation using computational tools such as **SIFT** and **PolyPhen-2** to assess the potential impact of these variants on protein function.

Variants existed at the same location in Ensembl database is rs758574181 (G/A allele a synonymous variant

SEMA6B GENE MUTATION ANALYSIS







Summary:

Three mutations were identified in all the samples. However, these variants do not match any recorded entries in the Ensembl database Ensembl genome browser 113. To establish these as novel variants, sequence more samples to confirm the consistency and validity of the variants and perform functional validation using computational tools such as SIFT and PolyPhen-2 to assess the potential impact of these variants on protein function**.**

KCNQ2 MUTATION ANALYSIS

Summary:

In the above table, we observed one type of mutation in two samples with heterozygous conditions with Synonymous variant means A synonymous variant, also known as a silent variant, is a change in the DNA sequence (a single nucleotide polymorphism, or SNP) that does not alter the amino acid sequence of the encoded protein because the new codon still codes for the same amino acid. c.1719C> T without changing amino acid with the location of rs61737409. With benign phenotype / Disease association.

The other gene mutations that were identified in the Whole Exome Sequencing didn't yield a positive result and further and hence additional extensive genetic studies are to be done for the children at higher risk of developing febrile convulsions.

DISCUSSION:

A pilot study of 12 samples was conducted and a Whole Exome Sequencing (WES) was sent to identify newer gene mutations.

The results of the yielded **KCNQ2, CPA61, SEMA6B, SAMD12, and ALH7A1 genes as positive** for febrile convulsions.

Febrile seizure (FS) mainly affected children in the 1–2 years age range according to our research findings and also affected another 40% of children who were between 2–5 years old. Current research matches up with findings that show FS occurs more frequently in children of age 1–2.

The research conducted in India by **Sharawat et al** demonstrated that sixty per cent of FS cases appeared among children within the age group of 6 to 24 months with an average age of 24.9 months. Upper respiratory infections were the most common cause of febrile seizures in children whose ratios were 2:1, according to the study findings.[46]

According to national registry data analysis from Korea, the highest frequency of FS occurrence was 27.5% within the age group of 2 years who were between 18–30 months old. Studies show the highest number of children experienced their first seizure in the second year of their life due to increased vulnerability. [47]

A study conducted in Nepal by **Agrawal et al** reported that males accounted for 70% of FS patients among which simple febrile seizures made up 48% while complex febrile seizures constituted 52%. The study recorded seizure recurrence in one-third of patients, particularly among individuals who were under 1 year of age and had male gender status. [48]

Research indicates both gender-based differences and age susceptibility in febrile seizures since most episodes occur before children turn two. Childhood febrile seizures return more frequently when children have their first seizure before reaching their first birthday. The comprehension of FS age-related patterns helps clinical professionals and caregivers prepare for and adequately manage seizures in young children.

Among the respondents, 38% had relatives with febrile convulsion history in their families while 62% experienced no such family history. The study participants provided information showing that 20% (n=10) of people with a family history of febrile convulsions had first-degree relatives who experienced febrile convulsions while another 16% (n=8) had febrile convulsions among second-degree relatives and only 4% (n=2) had third-degree relatives with such a history.

FS genetic factors play an essential role in seizure development because many participants show an inherited history of family seizures. The research did identify particular gene variations that enhance the risk of FS development.

A meta-analysis by **Yang X et al.** assessed the relationship between the GABRG2 rs211037 polymorphism along with FS development risk. The GABRG2 gene mutations produce either inactive or truncated γ2 elements of the GABA_A receptor, which cause subunit breakdown and reduce the surface expression of receptors. The alteration impairs GABAergic inhibition and may cause the development of epilepsy. The GABRG2 rs211037 mutation seems to regulate gene transcription and mRNA durability as well as protein translation efficiency, which affects susceptibility to FS based on the meta-analysis results. [49]

A research study by **Zare-Shahabadi et al.** focused on assessing single nucleotide polymorphisms (SNPs) of tumour necrosis factor-alpha (TNF-α) gene positions -308 and -238 within FS patient populations. Research results showed that TNF-α -238 GG genotypes

occurred more frequently in patients with FS as compared to the control participants. The data indicate that TNF-α SNPs function in the development of FS conditions. [50]

The research findings support previous studies by demonstrating that genetic variables consisting of family history together with genetic polymorphisms assist in determining who might develop FS. The occurrence of FS at higher rates among participants from families with a history of FS becomes a strong indication to recommend both genetic counselling and periodic checks for those individuals. Early detection through the identification of risk genes assists healthcare providers with managing susceptible children and reducing the impact of FS on patient families.

Our research indicated that typical febrile seizures occurred in 76% (n=38) of the participants, and atypical febrile seizures affected 24% (n=12) of the total participants. Half of the participants (40% or n=20) received clobazam while experiencing high temperatures.

The data shows typical febrile seizures exist as the most prevalent subtype consistent with established research findings. The features of typical febrile seizures include generalized tonic-clonic activity with a duration of less than 15 minutes that does not return within 24 hours but atypical febrile seizures have prolonged duration or focal characteristics or recurrent seizures in the same period.

Research data indicate that 40% of participants took clobazam while experiencing fever symptoms. Research indicates that clobazam provides preventive effects on febrile seizure recurrences through intermittently administered treatment during febrile illness periods.

Sattar et al. conducted research that analysed whether intermittent clobazam proved better than diazepam as a preventive treatment for recurrent febrile seizures. The results demonstrated that recurrent febrile seizures occurred in 9.4% of patients receiving clobazam, while 21.2% of patients under diazepam treatment experienced such seizures. Among the study participants,

clobazam proved better by generating fewer adverse effects such as drowsiness, sedation and ataxia than diazepam. The research determined clobazam exhibits efficiency when used as a treatment since it presents safety advantages while reducing its administration requirements compared to diazepam with fewer adverse effects. [51]

The research conducted **by Khosroshahi et al.** examined intermittent utilization of clobazam versus diazepam as an approach for stopping febrile seizures from returning. Seizure recurrence occurred in 1.7% of patients getting clobazam treatment versus 3.1% of patients getting diazepam treatment based on study results. The diazepam group showed drowsiness alongside sedation in 54% of cases yet only 14.2% of subjects taking clobazam experienced such adverse effects. This study determined intermittent clobazam to be superior to diazepam since it provided equal treatment outcomes though drowsiness and sedation adverse effects were significantly reduced. [52]

The research data shows clobazam provides effective seizure control as an intermittent prophylaxis for febrile seizures and patients tend to experience better tolerance of this treatment relative to diazepam. The limited appearance of negative effects associated with clobazam treatment leads to better adherence rates along with enhanced quality of life for both patients and their caregivers. Clinical findings indicate that 40% of participants received clobazam during fever episodes because medical professionals find the substance both effective and safe.

This research examined how particular gene variations occurred across Vijayapura district patients with febrile seizures (FS) located within Karnataka, South India. Data showed that three out of forty-nine patients (6%) tested positive for KCNQ2 mutations while one of forty-nine (2%) had CPA6 mutations, yet fifteen of forty-nine (30%) presented with SEMA6B mutations and only one patient with SAMD12 mutations (2%).

KCNQ2 Gene Mutations:

The rate of 6% of KCNQ2 genetic mutations found in our study population matches findings documented in scientific research about early-onset epileptic disorders. Results from Millichap et al. (2016) indicate that KCNQ2 mutations create an association between BFNS and early-onset epileptic encephalopathies. The range of KCNQ2 mutations shows that some individual mutations produce seizures that resolve independently while others trigger more serious conditions that delay development according to the authors. [53]

CPA6 Gene Mutations:

Our study confirmed the 2% occurrence rate of CPA6 mutations as originally reported by research that investigated FS and temporal lobe epilepsy (TLE). Salzmann et al. (2012) reported CPA6 gene mutations in a family with FS and TLE which indicated CPA6's role in seizure risk according to the authors. [54]

SEMA6B Gene Mutations:

A significant 30% of our studied patients presented SEMA6B gene variations. The association of SEMA6B genetic changes to the development of FS remains to be further confirmed. Neumann et al. (2021) documented SEMA6B gene mutation causing developmental delays alongside FS, which suggests the link between SEMA6B genetic variants and the development of seizure probability in the future for the patients. [55]

SAMD12 Gene Mutations:

Among the tested samples, our findings revealed SAMD12 mutations affecting 2% of patients, yet scientific evidence lacks sufficient connections between SAMD12 mutations and FS. Subsequent investigations need to establish any potential genetic relations between these factors.

CONCLUSION:

The extensive genetic diversity within FS requires patients to undergo complete genetic testing because researchers have proven its necessity through their examination. Research indicates that genetic mutations found in KCNQ2, CPA6 and SEMA6B genes affect susceptibility to FS in patients. More extensive research involving bigger participant groups should occur to both confirm these gene association findings and explain how these mutations cause FS.

Strengths:

1. The research gains a genetic understanding of febrile seizures (FS) by examining various gene polymorphisms through genomic evaluations of the Vijayapura district population.
2. Family history analysis as a part of the study helped to better understand genetic predisposition to disease.
3. The analysis through molecular genetics techniques evaluated genetic variations of key genes including KCNQ2, CPA6, SEMA6B and SAMD12 and added to existing research regarding FS genetic factors.
4. This work confirms its findings against previous literature research to demonstrate wider scientific value and reliability.

Recommendations:

1. Research needs to identify how the recognised gene mutations participate in the pathogenesis process.
2. Seizure recurrence patterns and risks related to genetic elements need to be measured through extended patient observation.

3. Demonstration centres across routine medical practices should implement genetic screening protocols to detect high-risk patients right after birth so they can receive proper management.

Limitations:

1. A small sample size hinders broad population generalization of the research outcomes.
2. The research examined specific genetic variations in one regional community thus researchers could not confirm whether these results apply to all ethnic population groups.
3. The study failed to accomplish functional testing on confirmed genetic variants thus leaving their actual mutation effects undetermined.
4. The study failed to analyse environmental and non-genetic risk factors during its investigation of FS development.
5. Extended genetic research on FS-related genes was not carried out due to lack of additional sequencing which restricted the field of genetic risk factors.

Conclusion:

Scientists discovered Febrile Seizure-related genetic polymorphisms that exist in KCNQ2, CPA6, SEMA6B and SAMD12 genes. The research revealed FS occurs as a heterogeneous condition within the population and genetic risk factors play an essential role in its development. This research investigation provides important findings for genetic epilepsy research which confirms that early genetic testing along with tailored preventive approaches should be conducted for people at high risk of genetic disorders.

Summary:

The childhood seizure disorder known as FS affects many patients and demonstrates high genetic roots in its pathophysiology.

Among FS patients we detected 6% KCNQ2 mutations together with 2% CPA6 mutations but SEMA6B mutations reached 30% and SAMD12 mutations were present in 2% of cases.

The genetic vulnerability for FS becomes stronger when patients have a strong family background linked to this condition.

The study verified that clobazam proved effective as an intermittent prophylactic medicine through treatment of 40% of participants according to existing published findings.

This study delivers essential genetic revelations though additional extensive functional investigations and bigger research efforts are necessary to verify findings and develop clinical applications.

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ANNEXURE-I

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT:

THE MOLECULAR CHARACTERISATION OF FEBRILE SEIZURES

PURPOSE OF RESEARCH:

I have been told that the present study will help in molecular characterization of febrile seizures.

PROCEDURE:

I do understand that after having obtained a detailed clinical history, thorough clinical examination and relevant investigations, a prospective study of febrile seizures.

RISK AND DISCOMFORTS:

I understand there is no risk involved and that the child may experience some pain and discomforts during the examination. This is mainly the result of the condition, and the procedures of this study are not expected to overemphasize these feelings, which are in association with the regular course of treatment.

BENEFIT :

I do understand that my participation in this study had no direct benefits to me, other than the potential benefit of the research and education.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subjected to confidentiality. Any information about sensitive, personal nature will not be a part of the medical record but will be stored in the investigations research file. If any of the data are used for publication in the medical literature or for teaching purpose, no name will be disclosed, and other identifiers such as photographs will be used only with special written permission taken priorly. I also understand that I may visualize the photograph before granting permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask questions about the study at any time; **Dr Chinmaya Rodgi** at the Department of Pediatrics is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr Chinmaya Rodgi may terminate my participation in the study after she has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my baby, resulting directly from baby's participation in this study; if such injury were reported promptly, the appropriate treatment would be available to the baby. But, no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have been explained about the purpose of the research, the Procedures required and the possible risks to the best of my ability.

_____ _____

Dr. Chinmaya Rodgi Date

(Investigator)

PARENTS / GUARDIAN CONSENT STATEMENT:

We confirm that **Dr Chinmaya Rodgi** is doing a study, "**The genetic polymorphism of febrile seizures**". A prospective follow-up study. Dr Chinmaya Rodgi has explained to us the purpose of the research and the study procedure. We are willing to give as much as information required for the study and consent for investigations and the possible discomforts as well as benefits. We have explained all the above in detail in our own language, and we understand the same. Therefore, we agree to give consent for the baby's participation as a subject in this research project.

_____ _____

(Parents / Guardian) Date

_____ _____

(Witness to signature) Date

[illegible] -586103

[illegible] / [illegible]

[illegible], [illegible] __________ [illegible], [illegible]/[illegible]/[illegible] __________ [illegible] _________ [illegible], [illegible] ___________, [illegible]/[illegible] _________ [illegible] ___________ [illegible] _________ [illegible] ______ [illegible] ([illegible]) [illegible]. [illegible]/[illegible]. [illegible] _______ [illegible] _______ [illegible]. [illegible]. [illegible], [illegible]. [illegible], [illegible].

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[illegible] ________________ [illegible]

[illegible]

[illegible]

1)

2)

ANNEXURE-II

PROFORMA

Name :

Age :

Sex :

Chief complaint :

Past history: significant / not significant, if significant specify

Birth history: significant / not significant, if significant specify

Antenatal history

Natal history

Postnatal history

Family history :

VITALS :

TEMPERATURE-

HR -

RR-

BP-

SYSTEMIC EXAMINATION :

CVS :

RS :

P/A :

CNS :

Diagnosis :

Investigations:

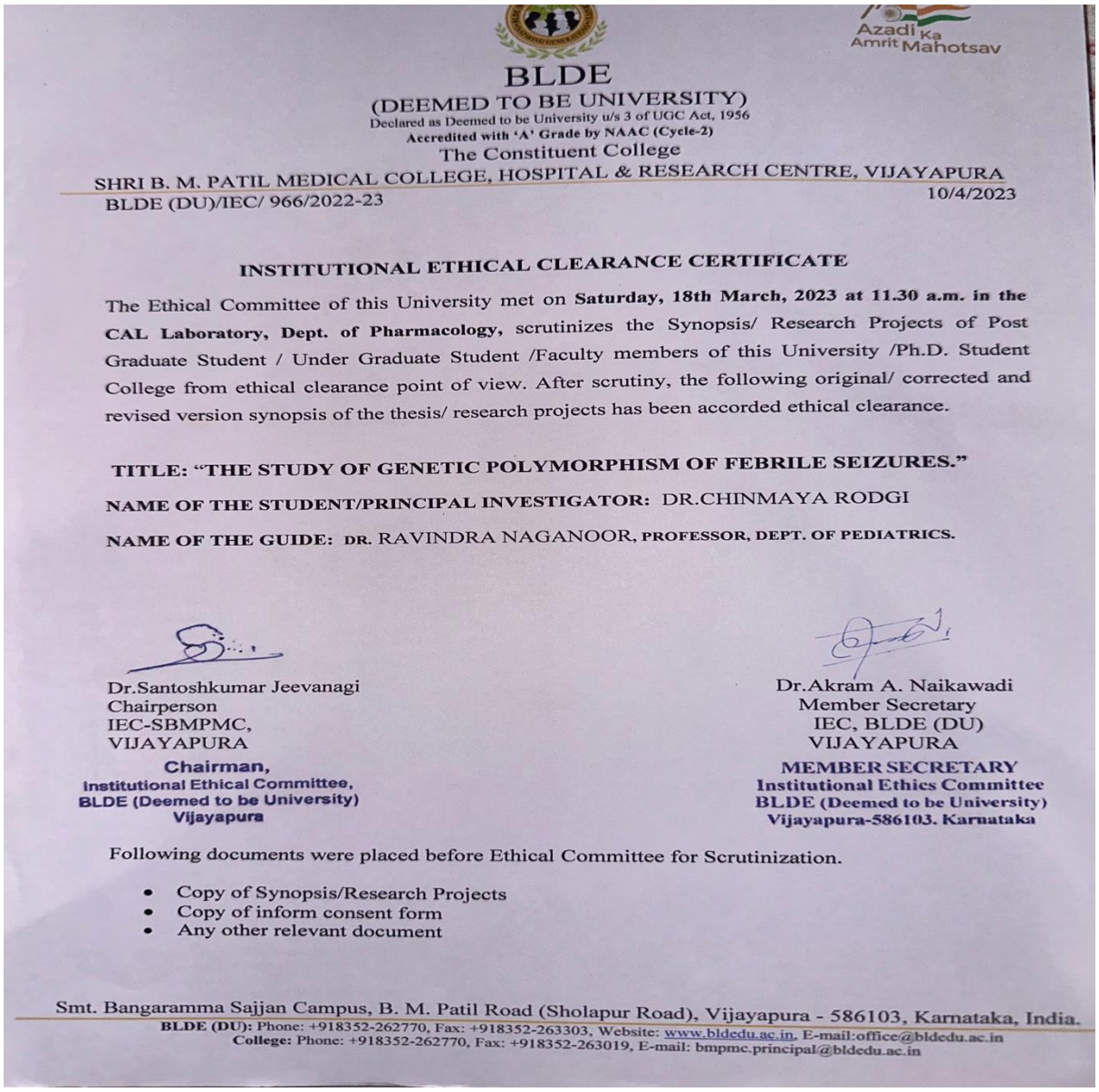
Duration of PICU stay :

Duration of hospital stay:

PROGNOSIS:

GENE NAME	**REPORT**
CPA6	
SEMA6B	
ALDH7A1	
KCQN2	
ALDH71	
SCN1A	
GABRA1	

ANNEXURE III



BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 966/2022-23 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "THE STUDY OF GENETIC POLYMORPHISM OF FEBRILE SEIZURES."

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.CHINMAYA RODGI

NAME OF THE GUIDE: DR. RAVINDRA NAGANOOR, **PROFESSOR, DEPT. OF PEDIATRICS.**

Dr.Santoshkumar Jeevanagi
Chairperson
IEC-SBMPMC,
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikawadi
Member Secretary
IEC, BLDE (DU)
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXURE IV

BIODATA OF GUIDE

NAME:	**Dr. RAVINDRA NAGANOOR**
DOB:	01/11/1972
EDUCATION:	MBBS, MD (PEDIATRICS)
KMC REGISTRATION NUMBER:	42799
WORK EXPERIENCE:	22 years. 11 years PG teaching
MEMBERSHIP	INDIAN ACADEMY OF PEDIATRICS
PRESENTLY WORKING AS	**PROFESSOR** DEPARTMENT OF PEDIATRIC SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL, BLDE (Deemed to be University) VIJAYAPURA – 586 103

ANNEXURE V

BIODATA OF CO GUIDE

NAME:	**DR GURUSHANTHAPPA S KADAKOL**
DOB:	01/03/1973
EDUCATION:	M Sc, PHD
WORK EXPERIENCE	7 YEARS
PRESENTLY WORKING AS	**RESEARCH SCIENTIST** GENETICS LABORATORY DEPARTMENT OF ANATOMY SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL, BLDE (Deemed to be University) VIJAYAPURA – 586 103

ANNEXURE VI

INVESTIGATOR BIODATA

NAME	: **DR CHINMAYA RODGI**
QUALIFICATION	: MBBS SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE B.L.D.E UNIVERSITY VIJAYPUR
REGISTRATION NO	: 149569
ADDRESS FOR CORRESPONDANCE	: DEPARTMENT OF PEDIATRICS SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE VIJAYAPUR – 586103.

ANNEXURE VII

MASTER CHART

Famil	Dat	E Birth	r On	Proj	On	Tak	On	Ant	Name of	Hear	Respi	Temper	Blood	Resp	Peri	Ce	Diagn	Total le	Different	Haem	Packed	Rel	Platelet	CR	Serun	Serum	Serum	Serum	Seru	Bloo	Lumb	If L	Duration	Dura	Prog	KCNQ2	GABRG2	CPA61	SEI	SAMI	ALH	SCN1A
No		2	Not si	Yes	Yes	Yes	Tablet cl		86	20	99.4	100/60	BILA	Soft,	Co	Typica	20,850	61134		9.8	29.8	4	1,38,00	6	0.9	142	4.2	9.8	100	62	Done	No	2 days	3 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
Yes	Sec	1	Not si	Yes	Yes	Yes			108	24	98.7	100/60	BILA	Soft,	Co	Typica	19,090	46147		6.9	18.8	3	2,19,00	<5	0.8	146	4.6	9.7	102	98	Not done		1 day	3 day	Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
No		2	Not si	No	No	No			82bp	24cpm	99.8°F	90/60m	BILA	Soft,	Co	Typica	14100	46144		9.8	28.6	4	3E+05	<5	0.6	142	4.6	9.1	116	92	Done	Normal		2 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
No		2	Not si	No	No	No			110t	24cpm	100	90/60	BILA	Soft,	Co	Atypica	9830	38.5/53.		11.2	32.9	4	5E+05	7	0.7	134	3.8	9.4	106	136	Done	No	1 day	3 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
Yes	Firs	1	Not si	Yes	Yes	Yes	Tablet cl		98	26	101.4	90/60	BILA	Soft,	Co	Typica	9590	54.2/35.		9.7	28	3	2,50,00	36	0.7	139	5.4	8.7	106	98	Not done		1 day	2 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
Yes	Firs	3	Not si	Yes	Yes	Yes			98	24	97.4	90/60	BILA	Soft,	Co	Atypica	11,600	74/12		9.8	29.4	4	2,62,00	<5	0.4	142	4.8	9.3	98	79	Done	No	1 day	2 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
No		1	Not si	No	No	No			98bp	28cpm	100.8°F	90/60m	BILA	Soft,	Co	Typica	11600	44.7/47.		12.3	31.6	4	5E+05	<5	0.5	134	3.2	8.2	104	136	Done	Normal		2 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
No		1	Not si	No		No			108	24	101.4	90/60	BILA	Soft,	Co	Atypica	16.09	57.8/36.		8.2	26.9	3	1,91,00	9	0.4	142	5.1	9	102	98	Not done		1 day	3 day	Good	Positive	Negative	Negative	Pos	Negy	Negy	Negative
No		1	Not si	No	No	No			98	26	101.4	100/60	BILA	Soft,	Co	Typica	20,950	61134.4		9.8	29.8	4	2,32,00	<5	0.5	146	4.2	9.1	96	110	Done	No	2 days	3 day	Poor	Negative	Negative	Negative	Pos	Negy	Negy	Negative
No		1	Not si	No	No	No			88bp	24cpm	98.6°F	90/60m	BILA	Soft,	Co	Atypica	54070	42/54.9		9.1	29.6	4	3E+05	9	0.6	142	4.6	9.2	113	98	Done	No	2 days	4 day	Good	Positive	Negative	Negative	Pos	Negy	Negy	Negative
No		2	Not si	No	No	No			98bp	20cpm	99.6	90/60m	BILA	Soft,	Co	Typica	5140	71/24		7.3	23.6	4	2E+05	9	0.7	138	5.49	9.3	102	150	Done	No	1 day	3 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative
No		1	Not si	No	No	No			94	20	98.7	100/60	BILA	Soft,	Co	Typica	9,000	44/40		11.2	30.1	4	2,36,00	7	0.3	146	4.6	9.3	102	94	Done	No	2 days	2 day	Good	Negative		Negative	Pos	Negy	Negy	Negative
Yes	Firs	2	Not si	Yes	Yes	Yes	Tab clob		92 bp	24 cpr	99.6	100/60	BILA	Soft,	Co	Atypica	10310	69.5/21.		11.4	33.5	4	3E+05	<5	0.5	143	4.2	7.8	105	110	Not done		1 day	2 day	Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
Yes	Sec	2	Not si	Yes	Yes	Yes	Tablet gu		108	24	98.7	100/60	BILA	Soft,	Co	Typica	11,700	72/24		11.8	32.2	4	2,48,00	6	0.8	140	4.2	9.3	108	98	Not done				Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
No		1	Not si	No	No	No			90bp	24cpm	98.7°F	90/60m	BILA	Soft,	Co	Typica	10310	69.5/21.		11.4	33.5	4	3E+05	13	0.3	137	4.6	8.8	114	96	Done	No	1 day	2 day	Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
Yes		1	Not si	Yes	Yes	No			84bp	24cpm	97.6	90/60	BILA	Soft,	Co	Typica	3630	33.3/64.		11.6	36.3	5	2E+05	56	0.4	132	0.4	8.9	132	126	Not done		2 days	4 day	Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
No	Sec	2	Not si	No	No	No			109t	26 cpr	98.7	90/60 r	BILA	Soft,	Co	Typica	6300	61/25.9		9.2	29.5	5	4E+05	19	0.3	137	4.2	9.2	106	134	Done	No	2 days	3 day	Good	Negative	Negative	Negative	Neg	Negy	Negy	Negative
No		2	Not si	No	No	No			110	20	98.7	90/60	BILA	Soft,	Co	Typica	19,820	38.3/53.		11.2	32.9	4	2,23,00	6	5.6	136	4.2	9.4	117	98	Not done		2 days	3 day	Good	Negative	Negative	Negative	Pos	Negy	Negy	Negative

ANNEXURE VIII

PLAGAIRISM CHECK

7% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text
- Small Matches (less than 10 words)

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