SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM, KERALA

GENOTYPE-PHENOTYPE CORRELATIONS AND PREDICTORS OF COGNITIVE OUTCOMES IN DRAVET SYNDROME & DRAVET BORDERLINE PHENOTYPES

Thesis submitted in partial fulfilment of the rules and regulations for

DM Degree Examination of

Sree Chitra Tirunal Institute for Medical Sciences and Technology

By

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Month and Year of Submission: August 2022

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2020-2022

DECLARATION

I, Dr. Krishna S, hereby declare that this project was undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology.

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Date: 13-08-2022

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The thesis entitled "**Genotype phenotype correlations and predictors of cognitive outcomes in Dravet syndrome and Dravet borderline phenotypes**" was carried out under my direct supervision. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

Clearance was obtained from the Institutional Ethics Committee for carrying out the study.

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This is to certify that thesis entitled "**Genotype phenotype correlations and predictors of cognitive outcomes in Dravet syndrome and Dravet borderline phenotypes**" is a bonafide research work done by **Dr Krishna S**, senior resident, Department of Neurology in partial fulfillment of the requirement for DM Neurology degree.

Date : 10/8/22

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ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to **Dr. Ramshekhar N Menon,** Professor of Neurology, SCTIMST, my guide for the study, for his expert guidance, constant review, kind help and keen interest at each and every step of the study. I am thankful to **Dr Ashalatha R**, Professor of Neurology and **Dr Soumya Sundaram**, Additional professor of Neurology, my co -guides for their valuable input and help for the study.

I am thankful to **Dr. Sylaja P.N,** Professor and Head, Department of Neurology, SCTIMST for her valuable input and help for the study.

I am thankful to the entire faculty of Department of Neurology, Dr Manna Jose, Mrs Alfiya F, nursing staff in Epilepsy unit and my colleagues for their valuable input and assistance to the study. I am extremely grateful to my family and friends for their overwhelming support and constant encouragement, especially Dr Reshma Rajan for helping me with the statistics.

I am greatly indebted to all my patients and their primary caregivers who participated in this study, without whose cooperation this study would not have been possible.

Above all, I thank The Almighty Lord, for His constant blessings.

Dr. Krishna S

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SYNOPSIS

Background and Purpose

SCN1A mutations have been associated predominantly with DS and GEFS+ spectrum and are characterized by marked phenotypic variability including age of seizure onset, seizure types and severity, as well as variable cognitive outcome. Genotype–phenotype correlations suggested are: truncating, nonsense, frame shift mutations, and partial or whole gene deletions are correlated with a classical DS phenotype and appear to have a significant correlation with an earlier age of seizure onset. Although it seems straightforward to infer that ‘‘severe’’ truncation mutations result in the more severe phenotype of DS and missense mutations cause GEFS+, this presumption is not always true as both SCN1A missense and truncation mutations were identified and that too in equal proportion (40% each) with DS patients. The same SCN1A mutations and deletions cause DS in some patients and GEFS+ in others, suggest that modifier genes, the genetic background, and/or environmental factors may also play a role in some patients, and thus DS may sometimes follow a complex model of inheritance. The severity of the phenotypes may also be correlated with the location of SCN1A missense mutations: for example, those in the pore-forming region of the sodium channel may cause DS, whereas missense changes associated with the GEFS+ spectrum may be more frequently located outside the pore-forming region. However, this genotype–phenotype correlation is not consistently observed. We aimed to ascertain electroclinical and cognitive phenotypic correlations of truncating and missense pathogenic/likely pathogenic variants of SCN1A mutations in DS and to determine developmental phenotypic features in relation to variants identified and pharmaco-responsiveness.

Materials and Methods

Ours is a single centre prospective study. Patients below 18 years of age who met the clinical criteria for Dravet syndrome or Dravet borderline phenotype, who had undergone genetic testing and had a minimum of one year follow up were selected for the study. All patients underwent neuropsychology, language, and occupational therapy assessment. The various parameters compared were DS vs DB, genetic test positivity vs negativity, missense vs truncating variants, seizure freedom vs ASM resistant epilepsy, seizure score <5 vs >5, family history of epilepsy or febrile seizures vs no family history, developmental delay vs normal development. We also tried to determine the predictors of seizure freedom and developmental delay.

Results

We had a total of 3967 paediatric epilepsy patients during the period 2015-2021, out of which 132 patients had fulfilled the clinical criteria for DS or DB, 62 underwent genetic testing and after excluding those whose reports were not ready by the time of analysis or who did not have a minimum 1 year follow up, 49 patients were selected for the study. There was no significant gender predominance in the cohort. Thirty seven (75.5%) had a positive genetic test, out of which 29 were SCN1A variants. There were 15 missense variants and 14 truncating variants. Among the variants 15 were pathogenic, 11 were likely pathogenic and 3 were promising VUS with phenotype matches. Among the 29 SCN1A variants, 23 were novel variants.

A non significant propensity was seen among typical Dravet phenotype towards febrile status, family history of febrile seizures or epilepsy whereas Dravet borderline phenotype had propensity towards clustering of seizures.

A trend towards typical DS was noted among children with truncating variants while DB phenotypes were more prevalent among children with missense variants. Multiple seizure types were also apparent among children with truncating variants and seizure freedom was more likely among children with missense variants although the differences were not statistically significant (p - 0.099)

No significant differences were noted among patients who tested positive and negative for variants following genetic testing.

A trend was seen for febrile seizures among the subgroup with seizure score $\geq$ 5. Those who had a later onset of unprovoked seizures were showing a trend towards seizure score $\geq$ 5. Any status epilepticus and multiple seizure types were noted among patient group with seizure score $\geq$5. Generalised IEDs were more favouring seizure score $\geq$5. Developmental delay, intellectual disability and learning disability were found to be significantly associated with seizure score $\geq$ 5 with p values 0.046, 0.028, 0.029 respectively. All 7 patients who were on Zonisamide were having seizure score $\geq$ 5 which was found to be significant (p- 0.028). Rest of the drugs were not found to have any significant association with seizure control.

Among those with and without seizure freedom, absence of febrile seizures was found to be significantly associated with seizure freedom (p-0.025). Those who had age appropriate development had significant association with seizure freedom. (p-0.022). Language delay and

learning disability were significantly associated with ASM resistant epilepsy (p-0.033 and 0.013 respectively), and there was a non significant propensity for intellectual disability and dependency in those with ASM resistant epilepsy. There was a trend for early age of onset of febrile seizures and multiple seizure types towards ASM resistant epilepsy. Multifocal IEDs in EEG, SCN1A positivity as well as truncating mutations also showed a trend towards ASM resistant epilepsy.

Developmental delay was significant in those who had febrile status when compared to those without febrile status (P- 0.029) and there was a trend seen with status epilepticus towards developmental delay. There was a trend towards developmental delay in patients with febrile seizures and in those with early age of onset of febrile seizures with clustering of febrile seizures. Multivariate analysis could not demonstrate any independent predictors of developmental delay.

Those who had positive family history had a later onset of febrile seizures than those with negative family history. Clustering of seizures were more seen in those without family history. Unprovoked clonic seizures were more common in those with negative family history (p-0.040) and myoclonic jerks were more common in those with positive family history (p-0.004) as expected, as Dravet phenotype were more common in those with positive family history. There was a non significant trend towards intellectual disability in those without family history.

Conclusion

This is the first study from the subcontinent which has addressed the significance of variant subtypes among a cohort of children who met the clinical criteria for DS or DB phenotypes. Apart from SCN1A variants we also detected SCN1B, GABRA1, GABRG2, PCDH19, SCN3A, CHD2, CACNA1H among others associated with DS or DB phenotypes. There is no electroclinical or radiological parameter to differentiate between the phenotypes of missense vs truncating mutations.

The severity of disease, seizure control, neurocognitive outcome does not vary between the two groups although children with missense variants demonstrated a trend towards Dravet borderline phenotypes, lower likelihood of multiple seizure subtypes and higher proportion were seizure free.

Our study establishes that all though truncating variants demonstrated a non significant trend towards more severe phenotypes the impact of severe missense variants in south India needs to be reiterated on seizure and developmental outcomes. Our results challenge the notion that truncating variants alone have a more severe impact on long term seizure and developmental outcomes among children with this developmental epileptic encephalopathy and SCN1A mediated epilepsy is a prototype DEE irrespective of the variant subtype.

Introduction

In 1978, Charlotte Dravet first described Severe myoclonic epilepsy of infancy (SMEI) and it was included in the International League Against Epilepsy (ILAE) classification in 1989, as an epileptic syndrome. (1) "Dravet syndrome" (DS) was proposed in the 2001 ILAE report and it included SMEI and "borderline" SMEI (SMEB). SMEB includes SMEI with atypical characteristics and low frequency.(2)

The prevalence of DS is found to be 1 in 20,000 to 1 in 40,000 members of the population, with a male-to-female ratio of 2 to 1. 3- 8% of patients who have their first seizure before 1 year of age have DS.(2)

According to 1989 revised ILAE classification, DS is characterised by febrile and afebrile seizures within 1 year of age in an otherwise normal child and the seizures can be generalised or unilateral clonic or tonic clonic and later on developing myoclonic seizures, atypical absences and partial seizures which are resistant to drugs and the child will later on have developmental regression, cognitive impairment and personality disorders .(1)

Mutations in the *SCN1A* gene which codes for the alpha subunit of the voltage-gated sodium channel 1.1, are found in more than 70% of patients with DS (SMEI and SMEB) (1–3). An observational study from our centre identified that the highest yield of genetic testing in terms of identification of pathogenic/ likely pathogenic variants (P/LP) is evident in DS and various well- defined as well as unclassified phenotypes of developmental epileptic encephalopathies of early infantile onset with a high likelihood of offering precision medicine based approaches with identification of ion channel variants (viz. *SCN1A*, *SCN2A*, *SCN8A*, KCNQ2, among others. (4)Prior studies have suggested the impact of truncating variants on the severity of the disease phenotype in DS from the seizure point of view however the developmental implications are uncertain. (5)Additionally the impact of arriving at a genetic confirmation in this group of patients while well-established from the prognostic as well as therapeutic point of view, direct comparisons between patients who have had a genetic confirmation versus those who have tested negative with available tests has not been attempted previously.

In this study we attempt to evaluate the phenotypic and electrophysiological correlates of various genetic variants and to determine the impact of various genetic variants on long term seizure and neurocognitive outcomes in DS and DB phenotypes. This is likely to be the first study from the subcontinent to assess the impact of both truncating and missense variants on a cohort of patients defined by stringent clinical and EEG criteria. .

The results of this study would enable therapeutic and prognostic modeling in a complex epilepsy syndrome i.e. DS which falls under the spectrum of developmental & epileptic encephalopathies (DEE) of childhood. The study can also potentially identify genotype-phenotype characteristics unique to India. A homogeneous cohort will also be identified who can benefit from potential targeted treatment intervention trials in the future. Functional predictive modeling of disease-causing variants based on position and types of mutations as well as amino acid change can also be strategized based on the results of the study based on functional predictions.

Review of Literature

Diagnosis of DS is based on age of onset, types of seizures and clinical course. DS presents with early tonic, clonic, tonic-clonic seizures which occur within the first year of life.(6) These seizures are usually associated with fever , and are often prolonged and generalized. Later on, patients can have afebrile seizures, including myoclonic, tonic-clonic, absence, simple and complex partial seizures.(6) In the initial stage, psychomotor and speech development will be normal, but developmental stagnation occurs by second year of life. Speech development will be delayed and patients often become ataxic.(6) There will be developmental regression and seizure intractability. (2)

The classical seizure types seen are febrile clonic seizures, myoclonic jerks, atypical absences and focal seizures with impaired awareness with convulsive, myoclonic or absence status epilepticus. Myoclonic jerks may not be seen in one – fifth of the patients. (7)

The first seizure occurs at around 5 to 6 months of age (can range from 2 to 10 months). It is usually a generalized or unilateral convulsion. Initial seizures are usually associated with fever and are typically prolonged, called as the preseismic period.(2) By the age of 2 years, the seismic stage starts where severe neurocognitive regression starts and multiple seizures are evident including focal or generalised myoclonus, atypical absences, atonia, complex partial seizures with automatisms, autonomic manifestations or obtundation status, which is characterised by fluctuating sensorium with reduced postural tone and myoclonic jerks.(2) The common triggers of seizures include fever, infections, hot bath or increase in body temperature, photic or pattern stimulation. (2) The post seismic stage is the one where frequency of seizures come down but with residual neurological and cognitive abnormalities. It usually starts at around 11-12 years. (7)

Myoclonic jerks usually start 1-2 years after onset of symptoms, can be segmental or generalised. Atypical absences occur in 40-93% and focal seizures in nearly 50 % of the patients. They can have adversion, atonic drops or automatisms.(7)

The development is usually normal before the onset of seizures, then it plateaus followed by a progressive decline which sets in between 1 to 4 years of age, usually in the second year of life. It can range from mild learning difficulty to global developmental delay. (2) DB patients however have a better outcome compared to DS patients. Ataxia and hyperreflexia are noted in some patients but those are not necessary to make the diagnosis. Family history of febrile seizures or epilepsy is seen in approximately 25% of cases.(1,2) A significant antecedent history is rare.

The clinical diagnosis of DS is supported by electroencephalogram (EEG), neuroimaging, and *SCN1A* mutation. EEG is usually normal at the onset, but it often progresses to generalized spike-and-wave discharges. A variety of interictal EEG findings are seen in these patients, some may have persistently normal EEG records.(2) Generalised and focal epileptiform discharges with early photosensitivity are seen in EEG. 40 % can have photoparoxysmal response, and this may be only abnormality in early stages. In two thirds of patients, there is progressive slowing within 1 year of illness with paroxysms of polyspikes or spike and slow waves. Sleep, eye closure and pattern stimulation can induce epileptiform discharges. Generalised spikes may not be seen in 10-15 % of the patients. (7) MRI brain are typically normal at onset of the disease. There can be mild cerebral or cerebellar atrophy or white matter T2 hyperintensity. (7) However, recent data shows that hippocampal sclerosis is frequently noted on further evolution of the disease.(8) Functional imaging can be normal or show focal hypoperfusion or hypometabolism. (7)

Mutations in the *SCN1A* gene which codes for the alpha subunit of the voltage-gated sodium channel 1.1, are found in more than 70% of patients with DS (SMEI and SMEB) (1–3). Mutations are dominant and can result in a clinical spectrum ranging in severity from the mild phenotypes of familial syndrome generalized epilepsy with febrile seizures plus to the severe phenotype of SMEI. (3) Mutations result in either a gain or a loss of function. Failure of inhibition leading on to excitation is the proposed mechanism in loss of function mutations in DS.(2)

The alpha 1 subunit of sodium channel has four homologous domains, and each domain has six transmembrane segments (S1-S6). These four domains are arranged around a central pore. The structural constituents of the central pore determine the selectivity and conductance properties of the channel. S5 and S6 segments and the S5-S6 linker line the permeation pathway, and these segments alone can be the functional pore. These segments also play a role in ion selectivity and generating electromotive forces required for electrical signalling (9)

Figure 3.1- Diagram of *SCN1A* gene

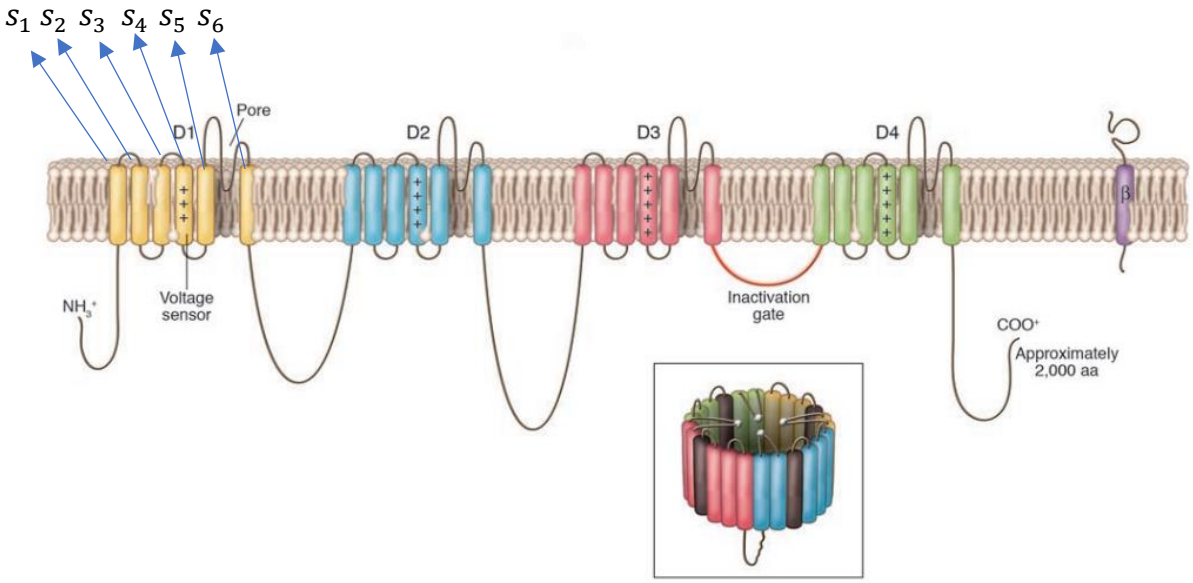


Diagram showing *SCN1A* gene with four domains, each domain having 6 transmembrane segments S1- S6. S4 has several positively charged amino acids and represents the voltage sensor. Pore loop has been labelled which lines the sodium ion permeable pore. Inactivation gate is also labelled.

The alpha subunits of mammalian voltage-gated sodium channel are encoded by nine genes, *SCN1A* through *SCN11A* excluding *SCN6A* and *SCN7A*, which code for non-voltage-gated sodium channel. The genes encode nine isoforms, Nav1.1 through Nav1.9. (10,11) Nav1.1, Nav1.2, Nav1.3 and Nav1.6 isoforms are expressed at high levels in the CNS and Nav1.6, Nav1.7, Nav1.8 and Nav1.9 isoforms are expressed at high levels in the peripheral nervous system. Nav1.4 isoform is expressed predominantly in adult skeletal muscle and Nav1.5 in embryonic skeletal muscle and heart muscle. (11)

In adults, Nav1.1 is the predominant channel in the caudal regions of brain and the spinal cord, present at high levels in dendrites and cell bodies whereas Nav1.2 levels are highest in the rostral regions, dendrites and unmyelinated axons. (11,12) Nav1.6 is present in dendrites, cell bodies, axons, pre- and post-synaptic sites. It is the main sodium channel at the nodes of Ranvier. (11,13)

The voltage-gated sodium channel is responsible for the initiation of action potentials and, thus involved in neuronal excitability. Action potentials are initiated preferentially at the initial segment of distal end of the axon compared to the proximal end. This is due to the high

density of Nav1.6 channels in the distal end and it has a lower threshold for activation whereas in the proximal end Nav 1.2 channels are high. (11,14)

More than 170 documented mutations are associated with SMEI and SMEB. Nearly 50 % of the them are truncation mutations, with the remainder being missense, splice site and deletion mutations.(3) Mutation analysis using PCR-based sequencing identified point mutations, small deletions, and insertions. These were de novo in about 30 to 70% of SMEI patients. In the remaining patients, other types of mutations like microdeletions comprising the gene, mutations in regulatory sequences, or mutations in other genes were identified.(15) Intragenic and whole gene deletions were identified in a few cases of SMEI without truncation, missense or splice-site mutations (3) However, at least 20% do not have detectable mutations in any of the known genes, including *SCN1A*.(16)

Truncation mutations are usually said to cause a severe disturbance in protein functions than missense mutations and almost all the reported truncation mutations of *SCN1A* having resulted in the phenotype SMEI, SMEB, or intractable childhood epilepsies with frequent generalized tonic clonic seizures (ICEGTC).(9) Missense mutations, however are identified in SMEI, SMEB, ICEGTC as well as in generalized epilepsy with febrile seizures plus (GEFS +),which is an autosomal dominant epilepsy with febrile seizures in children, which often persist beyond 6 years of age and afebrile seizures in adults. (9)

Missense mutations in the S5-S6 pore forming regions of *SCN1A* are found to be associated with a more severe phenotype (SMEI with ataxia and early disease onset) than mutations in other regions.(3,9) One probable explanation for this is that the mutations in the pore regions may produce more severe channel dysfunction like activation/inactivation dysfunction or abnormal voltage dependency as compared to mutations in other transmembrane regions, thus determining the phenotypic presentation. (9)

In reality, the phenotypic corelation with the various types of mutations is impossible to predict as patients with the same variant can cause a variety of phenotypes, even with the same genetic background. (17)

Mutations in *SCN1A* (Nav1.1) and *SCN2A* (Nav1.2) can cause several subtypes of dominant idiopathic generalized epilepsy, genetic epilepsy with febrile seizures plus (GEFS+). *SCN1B* gene, which encodes the sodium channel β1 subunit, *GABRG2* gene, which encodes the γ2 subunit of GABA receptor and *GABRD* gene, which encodes the δ subunit of GABA receptor are also responsible for GEFS +. (11,18–20) *SCN1A* mutations are the major cause for Dravet

syndrome and ICEGTC. (21) *SCN2A* and *SCN1B* mutations have also been identified in DS. (22,23) SCN9A might serve as a genetic modifier of DS and its mutations were identified in febrile seizures. (24) Other mutations identified were *PCDH19* and *STXBP1*. (25) Recently *HCN1* gene mutations are known to present with phenotypes similar to DS. (26)

The variability in clinical severity among patients carrying the same mutation is very striking in GEFS+ and SMEI. Several factors can contribute to these differences in phenotype. There is an intrinsic stochastic variability in the developmental processes. These are especially important during the development of neuronal connectivity in the central nervous system.(27) Another explanation which has been suggested is that due to the accumulation of somatic mutations in the lifetime of an individual, it can affect the development of neurological disease like epilepsy.(28) Environmental insults can also exacerbate the clinical expression in people who carry a mild mutation. Lastly , the various differences in genetic background together with various other susceptibility factors can contribute to the varied clinical severity in patients. (27)

Germline and somatic *SCN1A* mutational mosaicism were reported in unaffected or mildly affected parents of children, who have SMEI or SMEB.(3,29) The uncovering of mosaicism could be a plausible explanation for the reduced penetrance, which was previously observed in several parents of SMEI children.(29) The idea of mosaicism in Dravet syndrome has so many implications in genetic counselling, as the potential risk of recurrent transmission can be estimated only when the mutated allele is detected in the blood of the parent and cannot be predicted early in apparently de novo cases. This has been demonstrated in families wherein disease phenotypes have been noted to occur in siblings and postulated to be due to gonadal mosaicism of paternal origin.(30)Therefore, the risk of recurrence in families with apparently de novo SMEI cases might not be as low as estimated previously.(29)

Refractory seizures are a hallmark of DS. Carbamazepine, oxcarbazepine, phenytoin and lamotrigine should be avoided in DS as those can cause exacerbation of seizures. Valproate, topiramate and levetiracetam are usually given as the first line agents.(2) Stiripentol, an inhibitor of cytochrome P450 is particularly effective against status epilepticus. It increases GABA A receptor channel opening duration as well as interfere with GABA reuptake and metabolism, thereby enhancing GABA neurotransmission. It is the only drug which is approved in Dravet syndrome in combination with valproate and clobazam and it is found to be effective in reducing the duration and frequency of seizures in Dravet syndrome.(31)

Cannabidiol was studied as an add on drug in DS and it was found to be effective in the long term reduction of seizure frequency in refractory DS. The exact mechanism of action is not known, but it enhances GABA activity through allosteric modulation of GABA A receptor. (32) Fenfluramine was also found to effective in refractory DS. It increases serotonin concentration by disrupting its vesicular storage and reversing serotonin transporter function.(33)

Ketogenic diet (KD), started at early stages has shown favourable outcome. KDs include classical KD, which is a high fat low carbohydrate (fat : carbohydrate + protein = 3-4:1), medium chain triglyceride diet, modified Atkins diet and low glycemic index treatment. The exact mechanism of KD is not clear but the purpose is to mimic starvation such that ketones will be used up as the primary energy source, which in turn induce anaplerosis in mitochondria and favour antioxidant synthesis and corresponding metabolic changes has antiepileptic effects. (34)

Observations showed that, certain mutations have therapeutic implications, like some nontruncating mutations might impair trafficking of NaV1.1 to the plasma membrane and drugs like phenytoin and lamotrigine can increase the cell surface expression and thus these sodium channel blockers may be paradoxically amenable to pharmacological rescue in DS. (35) Recently anti-sense oligonucleotides which lead to a selective mRNA based transcription of the wild type (WT) *SCN1A* gene and reduce the expression of the mutant allele of the *SCN1A* gene has been found to reduce the seizure burden and prevent SUDEP.(36)

Avoidance of triggers is very important in preventing seizures in DS. Measures like avoiding hot baths or using cooling vests in hot weather can be tried if the patient is sensitive to hyperthermia. Sunglasses can be used if the patient has photosensitivity. Benzodiazepine should be kept handy and can be used in the home to prevent status epilepticus. (2)

Outcome is poor in DS. Usually after 4 years of age, patients reach a steady state of intractable seizures, impaired intellect, behavioural disturbances, and neurologic abnormalities. Myoclonic seizures usually subside and are replaced with absence seizures or nocturnal generalized clonic seizures. The number of seizures determines the degree of developmental regression.

Genetic testing is very useful in Dravet syndrome such that it establishes the etiology, so we can avoid further diagnostic tests and procedures, allows early optimization of antiseizure

medications(ASM), carry out an aggressive management and it also helps in counselling. But it's not useful in GEFS + where there is extensive phenotypic heterogenicity and the presence of mutation will not predict the prognosis and it has no effect on the treatment protocols. (37)

If there are multiple affected family members with GEFS+, a positive test for *SCN1A* mutation can predict occurrence of seizures in an individual, but the clinical outcome would range from typical febrile seizures without any cognitive decline or further seizures to severe Dravet syndrome. In GEFS+, the penetrance of missense mutations in *SCN1A* is only 60–70%, hence all mutation carriers will not develop seizures. Here, diagnostic genetic testing has greater utility than predictive testing. In the future, predictive testing can be utilised when all of the genes which influence the clinical outcome are identified, which needs complex protocols for testing multiple genes and understanding how these genes interact causing their influence on risk. As more than 70% of Dravet syndrome have *SCN1A* mutations and among them, more than 95% are de novo mutations, the clinical sensitivity of a diagnostic genetic test is high regardless of the family history. (37)

The mortality rate is DS is around 16% and is usually caused by prolonged convulsive seizures, drowning or sudden unexpected death.(2)Sudden unexpected death in epilepsy (SUDEP) defined as the sudden death in a person with epilepsy in the absence of an obvious cause of death, is a rare complication of epilepsy. The risk of SUDEP in Dravet syndrome is around 15 times more than other childhood epilepsy syndromes. This can be a major concern for the families. Prior studies have observed that *SCN1A* mutation correlated with ictal bradycardia in mouse models. Thus, parasympathetic modulation can be explored as an option to limit duration of bradycardia and thereby improving the SUDEP risk.(38)

Objectives

Aims and Objectives

Primary Objectives

- To ascertain electroclinical and cognitive phenotypic correlations of truncating and missense pathogenic/likely pathogenic variants of *SCN1A* mutations in DS
- To determine developmental phenotypic features in relation to variants identified and pharmaco-responsiveness
- To determine impact of the subtype of *SCN1A* variants on long term seizure outcome in DS and DB phenotypes

Secondary Objectives

- To determine correlates of other pathogenic variants in children with DS
- To elaborate distinctive features of children who meet clinical criteria for DS/Dravet borderline phenotypes who have no identified variants on genetic testing.

Methodology

This was a prospective cohort study.

All patients diagnosed with Dravet syndrome and newly diagnosed cases during the study period (2015-2021) attending the neurology wards and outpatient department, Department of Neurology, SCTIMST fulfilling the inclusion and exclusion criteria were selected.

Case definition

Typical DS was defined based on seizure onset usually in the first year of life of convulsive seizures which should include all of the following

- Onset usually in the first year of life of convulsive seizures which should include: a) fever-provoked or unprovoked hemiclonic or generalized; b)myoclonic seizures; c) other seizure types which could include focal seizures, absence seizures, atonic seizures, tonic seizures
- Normal development in the first year of life with subsequent slowing including plateauing or regression
- Generalized spike-wave activity on EEG and normal MRI or non-specific findings.

Dravet borderline (DB) phenotype

- Absence of specific features that are regarded as required for the diagnosis of DS:
- DB-M- patients who did not have myoclonic seizures but otherwise satisfied DS criteria.
- DB-SW- patients who met all the DS criteria but never had generalized spike-wave activity documented on EEG.
- DB-O- patients who had more than one feature that was not in keeping with DS; eg. absence of generalized spike-wave activity recorded on EEG, a normal developmental outcome and absence of myoclonic seizures.
- DB-GTCS – Children with only intractable generalized tonic clonic seizures (GTCS) and followed the same course as DS

Inclusion criteria

Patients fitting into the case definition within the age group of 6 months to 18 years (paediatric age group).

ii) Should have undergone genetic testing with MLPA/ clinical exome sequencing (CES)/ *SCN1A* sequencing which is mandatory and part of the standard of care for diagnosis of DS.

iii) For newly diagnosed patients (based on electro-clinical features) should agree to undergo genetic testing as part of their standard of care.

iv) Should consent for formal neuropsychology, speech-language and occupation therapy assessment .

v) Should consent for at least 6 monthly clinical follow-ups/ telephone based follow up.

vi) Subjects who meet the core criteria for DS and evolve as other epileptic encephalopathies such as LGS or encephalopathy with continuous spike and wave during sleep will also be included.

Exclusion criteria

i)Absence of genetic test results or who do not consent for genetic testing to be conducted as per standard of care after pre-test counselling.

ii)No formal neuropsychology, speech language, OT assessment done.

iii)Significant perinatal insult defined as APGAR <6 at 5 minutes associated with neonatal encephalopathy or significant developmental delay as assessed on revised Denver developmental scale prior to seizure onset

iv)Not meeting criteria for DS as mentioned above.

We screened all clinical, EEG, imaging and genetic testing records to identify cases that fulfil the diagnostic criteria for Dravet syndrome. Clinical records were studied to find out the antecedent illness, family history, vaccine triggers, course of the illness, intellectual, cognitive and psychological development and treatment details as well as the final status on the last visit. Structured proforma (appendix 1) was designed to obtain electro-clinical data on all patients with specific emphasis on early seizure history including age of onset, occurrence of fever provoked or unprovoked status epilepticus, number of hospitalizations for seizure

clusters or status epilepticus, presence of fever sensitivity, clinical photo-sensitivity, profile of pharmaco-responsiveness and evolution of other seizure types as well as last available seizure status.

A detailed early developmental history was obtained with attention to acquisition of early milestones, timing of plateau or regression of development and current functioning. Other important details included general and neurological examination, family history of seizure disorders and results of EEG, video-EEG monitoring and MRI Brain all of which are routinely done as part of standard of care.

Detailed neurocognitive assessment was done on follow up. Scales used were Denver II Development Screening Test for developmental age, Vineland Social Maturity Scale- Indian version (raw and scaled scores) and Wechsler Intelligence Scale for Children (raw and scaled scores for children > 6 years for diagnosis of intellectual disability. Developmental outcomes at last available follow up were graded using DDST based on DA/CA * 100 with grades of mild, moderate and severe for DQ grades of 50- 70%, 49-30%, <30 % respectively.

Genetic test results available from the genetic labs where the tests are carried out- (commercial and potential in-house when available) was stratified according to ACMG (American College of Medical Genetics and Genomics) criteria as class 3-5. Variants are divided into 5 classes. Class 1- benign, Class 2 likely benign, Class 3 variant of uncertain significance (VUS), Class 4 likely pathogenic, Class 5 pathogenic.

Once the VUS identified on NGS in the *SCN1A* gene is confirmed by Sanger sequencing, the lab usually recommends segregation analysis on parents' samples to establish if the variant is denovo. In most situations as parents cannot afford testing, this is not done. We did not order segregation analysis specifically as part of this study and the variants were maintained as a VUS if characterized as such under the ACMG criteria. In routine practise, only if the patient's parents can afford and consent for parental testing is the segregation analysis ordered as per the wishes of the parents. During genetic counselling, they are counselled that *SCN1A* variants have incomplete penetrance and variable expressivity within families given the autosomal dominant nature of inheritance and parental testing will only identify if the VUS is a de novo one or not. However online databases used to characterize these VUS are frequently updated and it is possible that data will emerge in future on the pathogenicity of the variant based on insilico predictions or invitro functional assay models. (Consent form – Appendix 2)

Functional effect predictions using in-silico tool assessment information provided by the genetic labs were documented. The final interpretation of the genetic lab was documented in accordance with ACMG criteria and there was no attempt on the investigators side upon receipt of the results to modify this classification. However, for VUS variants, we have reviewed the online databases to know whether pathogenicity has been established. In case of novel variants identified, in-silico predictions were relied upon including the results of segregation analysis if done by the lab. Gain of function versus loss of function or mixed effects was documented for those whose data were available.

For speech assessment, REELS (Receptive Expressive Emergent Language test) was administered; Occupation therapy assessment for ADL (Activities of daily living), functionality and education progress were also documented.

Co-morbid cognitive-behavioural outcomes such as Autism was diagnosed using DSM V (Diagnostic and Statistical Manual of Mental Disorders) and ADHD (attention deficit hyperactivity disorder) using DSM V.

Cognitive and psychosocial profiles assessments were done during clinic visits. Clinical and developmental age assessments was done at last follow up. The minimal follow up duration was 1 year.

Institute ethics approval was obtained (IEC/1595)

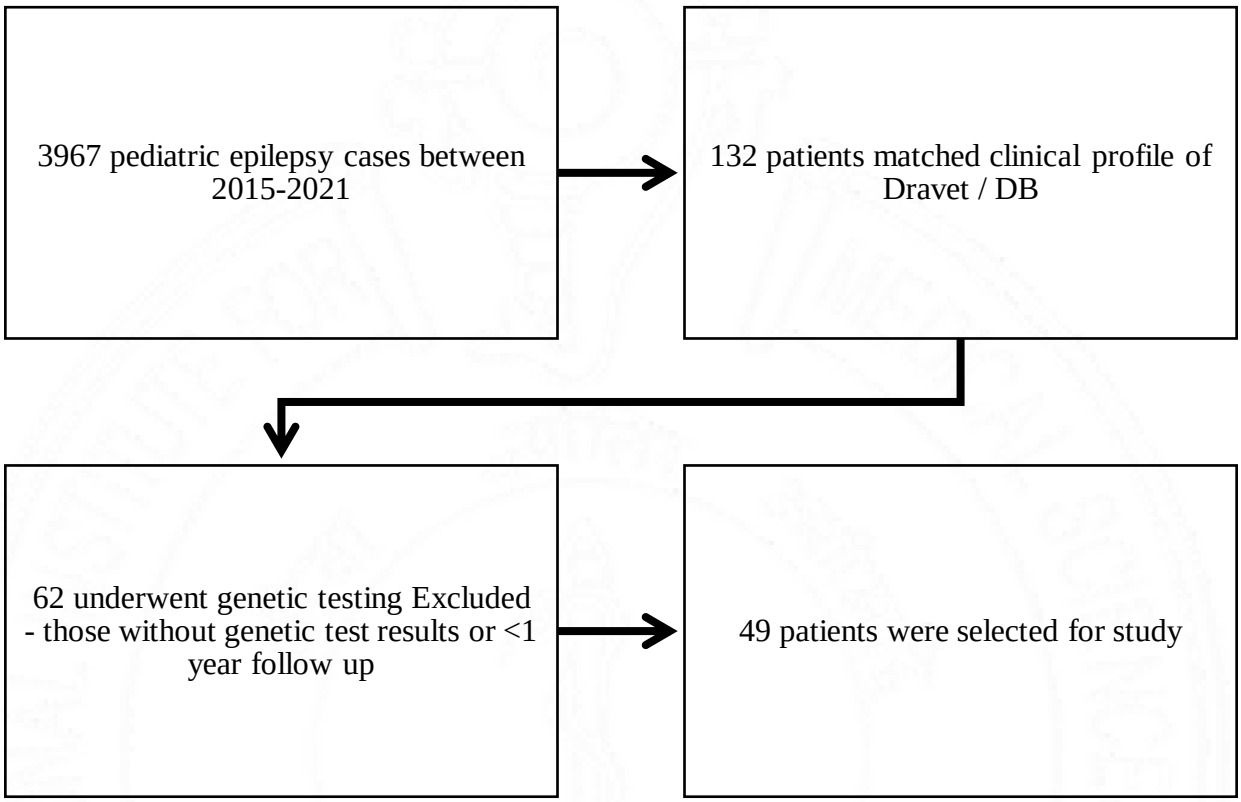
Statistical analysis:

Data was transferred to Excel sheets and analysed with SPSS software. Descriptive variables were analysed with Mean, standard deviation and Proportions. Qualitative variables were compared using Fisher exact test and quantitative variables using ANOVA. T test was be used for testing mean comparison and Chi square test for testing association. Comparisons were made between seizure free versus non-seizure free; seizure score <5 vs >5, truncating versus missense; genetic test positive versus negative; DS versus DB phenotypes, positive family history vs negative, normal development vs delayed development.

Multiple logistic regression analysis was done to ascertain impact of various phenotypic and electrical variables on developmental outcome.

Results

The flow diagram below summarizes the strategy employed for recruitment of patients into the study.



Among the 49, 53.1% were males and 46.9% were females. Clinical follow up ranged from 1-17 years with a median duration of follow up in our registry being 3years.

Table 6.1- Demographic data

Demographic variables	**Number of patients (%)**
Gender – male	26 (53.1%)
Female	23 (46.9%)
Consanguinity	1 (2 %)
Sibling affliction	3 (9.1%)
Mode of delivery – vaginal	21 (42.9%)
LSCS	22 (44.9%)
Assisted vaginal	6 (12.2%)
Antenatal period	
GDM	2 (4.1%)
PIH	3 (6.1%)
Preterm delivery	3 (6.1%)
Perinatal complications	7 (14.3 %)
Respiratory distress	2 (4.1%)
Neonatal jaundice	2 (4.1%)
Neonatal sepsis	1 (2 %)
Others	2 (4.1%)
Delayed cry	3 (6.1 %)
NICU admission	7 (14.3 %)
Ventilatory care	1 (2 %)
Poor feeding	2 (4.1%)

(Abbreviations explained in Appendix 3)

Only 1 patient was born of third-degree consanguineous parentage, he had intractable epilepsy and developmental delay. However, he did not have family history of febrile seizures or epilepsy.

Twenty (40.8 %) had delayed development at enrolment and 9 (18.4%) had regression in one or more domains, diagnosed using Denver Developmental Screening Test. Four (8.2%) had autism spectrum disorder at enrolment, diagnosed using DSM-V criteria.

Table 6.2- Antecedent data

Clinical variables	Number of patients (%)
Developmental delay	20 (40.8 %)
Global	13 (26.5%)
Motor delay	5 (10.2%)
Language delay	2 (4.1%)
Any Regression	9 (18.4%)
Global	5 (10.2%)
Language	4 (8.1%)
ASD	4 (8.2%)
Family history of seizures	24 (48.9%)
Febrile seizures	17 (34.7%)
Epilepsy	6 (12.2%)
Both FS and epilepsy	1 (2%)
Speech abnormality	20 (40.8%)
Nonspecific dysarthria	14 (28.6%)
Cerebellar	1 (2%)
Non verbal	5 (10.2%)
Ataxia	10 (20.4%)
Axial	7 (14.3%)
Appendicular	1(2%)
Both	2 (4.1%)
Gait abnormality	11 (22.4%)
Ataxic	8 (16.3%)
Non ambulant	3 (6.1%)

(Abbreviations explained in Appendix 3)

On examination, 5 (10.2%) had neurocutaneous markers – café au lait spots, 3 (6.1%) had facial dysmorphism (2 had epicanthal folds and 1 had hypertelorism), 20 (40.8%) had speech abnormalities. 1 had microcephaly, 2 had hypotonia, none had spasticity, 3 had pyramidal signs, none had extrapyramidal signs, 10 (20.4%) had ataxia.

Table 6.3- Seizure subtypes based on history and video descriptions where available

Seizure variables	Number of patients (percentage)
Febrile seizures	45 (91.8%)
Simple FS	11 (22.4%)
Complex FS	34 (69.3%)
Febrile seizure frequency	
1-11/year	16 (35.5%)
Every month	15 (33.3%)
Every week	14 (31.1%)
Clustering of febrile seizures	30 (61.2%)
Febrile status	20 (40.8%)
Medication used for febrile status	
Benzodiazepine	6 (12.2%)
Valproate	1 (2%)
Phenytoin	1 (2%)
Levetiracetam	1 (2%)
Phenobarbitone	1 (2%)
Unknown	10 (20.4%)
Semiology of febrile seizures	
Focal motor with awareness	1 (2%)
Focal motor without awareness	27 (55%)
Focal non motor with awareness	1 (2%)
Focal non motor without awareness	2 (4.1%)
Focal to bilateral tonic clonic	13 (26.5%)
Generalised motor	23 (46.9%)
Generalised non motor	2 (4.1%)
Unclassified	1 (2%)
Febrile motor seizures	
Tonic	7 (14.3%)
Clonic	24 (49%)
Tonic clonic	34 (69.4%)
Myoclonic	1 (2%)
Atonic	1 (2%)

Unprovoked seizures	49 (100%)
Focal motor without awareness	37 (75.5%)
Focal non motor with awareness	2 (4.1%)
Focal non motor without awareness	5 (10.2%)
Focal to bilateral tonic clonic	14 (28.6%)
Generalised motor	25 (51%)
Generalised non motor	2 (4.1%)
Unprovoked motor seizures	
Tonic	21 (42.9%)
Clonic	27 (55.1%)
Tonic clonic	34 (69.4%)
Myoclonic	26 (53.1%)
Atonic	5 (10.2%)
Absences : Typical	3 (6.1%)
Atypical	2 (4.1%)
Clustering of unprovoked seizures	26 (53.1 %)
Unprovoked status	9 (18.4 %)
Vaccine provoked seizures	22 (44.9%)
DTP	11 (22.4%)
Pentavalent	5 (10.2%)
IPV	1(2%)
Measles	1(2%)
Unknown	4 (8.1%)
Timing of seizures	
Same day	20(40.8%)
Same week	2 (4.1%)
Any clustering	42 (85.7 %)
Any status	24 (49 %)
Multiple seizure subtypes ($\geq$ 2)	39 (81.6%)
Epilepsy classification	
Focal	19 (38.8%)
Generalised	7 (14.3%)
Combined	23 (46.9%)

Lateralisation based on semiology	
Right	2 (4.1%)
Left	6 (12.2%)
Bihemispheric	21 (42.9%)
Uncertain	14 (28.6%)
Localisation based on semiology	
Uncertain	35 (71.4%)
Frontal	3 (6.1%)
Occipital	3 (6.1%)
Sleep activation of seizures	7 (14.3%)

(Abbreviations explained in Appendix 3)

Complex febrile seizures predominated among the febrile seizures. Focal motor seizures without awareness were the most dominant among febrile seizures as well as unprovoked seizures followed by generalised motor seizures. Tonic clonic seizures predominated among the motor seizures followed by clonic seizures. Clustering of seizures were seen in more than half of the cohort. Vaccine provoked seizures were seen in less than half of the cohort, but in those who had, seizures occurred on the same day as vaccination in > 80%. Combined epilepsy was dominant epilepsy in the cohort and majority had bihemispheric lateralisation based on history, though localisation was not certain. A small percent had sleep activation of seizures.

The prevalence of multiple seizure subtypes (2 or more) was 81.6% in this cohort. 87.8 % had focal seizures and 87.8 % had generalised seizures.

Twenty one (42.9%) had undergone cerebrospinal fluid study out of which 80% had a normal study and remaining had pleocytosis with raised protein.

Table 6.4- EEG data

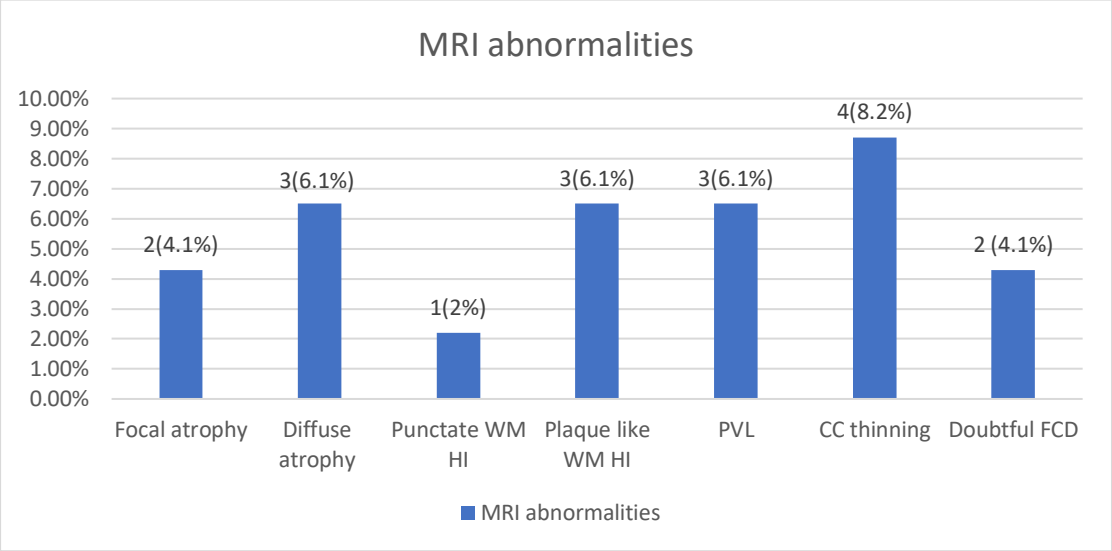
EEG variables	Number of patients (percentage)
Abnormal EEG	44 (89.8%)
Background activity	
Normal	28 (57.1%)
Focal slowing	8 (16.3%)
Diffuse slowing	11 (22.4%)
Hemispheric slowing	2 (4.1%)
Location of IEDs	
Right	3 (6.1%)
Left	3 (6.1%)
Bilateral	35 (71.4%)
Location of IEDs	
Frontal	14 (28.5%)
Temporal	1 (2%)
PHR	3 (6.1%)
Centrotemporal	2 (4.1%)
Centroparietal	1 (2%)
Frontotemporal	4 (8.2%)
Frontal and PHR	3 (6.1%)
Multifocal IEDs	13 (26.5%)
Generalised IEDs	17 (34.7%)
GPFA	1 (2%)
Photosensitivity	2 (4.1%)
Activation in sleep	
Normal	2 (4.1%)
Continued activation	30 (61.2%)
Marked activation	7 (14.3%)
Activation only in sleep	4 (8.2%)
Sleep architecture	
Well formed	46 (93.8%)
Poorly formed	2 (4.1%)
Absent	1 (2%)

Posterior dominant rhythm	
Age appropriate	40 (81.6%)
Slow for age	9 (18.4%)
VEEG - seizures recorded (5)	
Focal seizures	2 (4.1%)
Focal seizure with secondary generalisation	3 (6.1%)
VEEG – ictal pattern (5)	
Fast recruiting rhythm	3 (6.1%)
Spike and wave	2 (4.1%)
VEEG – ictal onset	
Right	2 (4.1%)
Left	1 (2%)
Bilateral	2 (4.1%)
Generalised	1 (2%)

(Abbreviations explained in Appendix 3)

EEG was abnormal in around 90% of the cohort, more than half had normal background activity. Majority had bilateral epileptiform discharges with frontal predominance. Multifocal and generalised discharges were seen in less than half of the cohort. Sleep EEG was abnormal in 96 % of the study population. However, only one patient had history of sleep disturbances (insomnia). Sleep architecture was well formed in > 90 % with > 80 % having age-appropriate posterior dominant rhythm. Only 5 patients had recordable events in VEEG.

Figure 6.1 MRI abnormalities



Sixteen patients (32.7%) had MRI abnormalities. Probable FCD was reported in 2 of the patients, both had perinatal insult: 1 had respiratory distress and the other had pathological jaundice. They had focal hypoperfusion in ASL without any obvious parenchymal lesion. EEG showed bilateral frontal and generalised IEDs and recorded events showed bilateral ictal onset in both the patients which was not suggestive of FCD.

Figure 6.2 Current anti-seizure medications

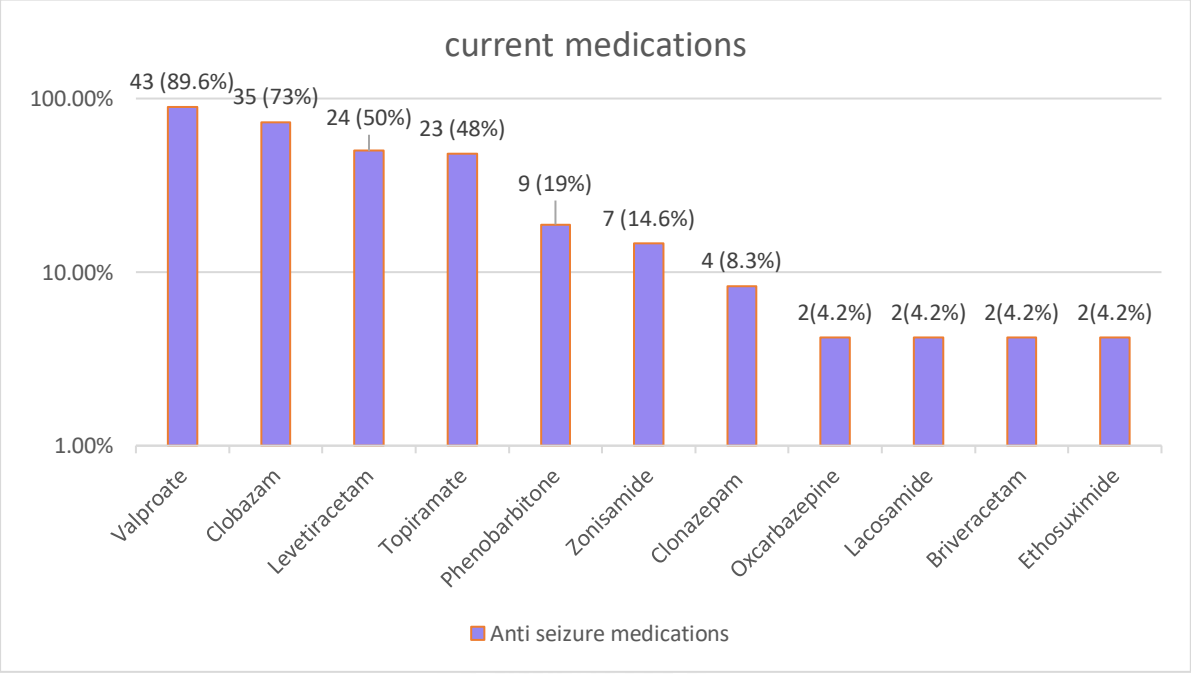


Figure shows the antiseizure medications on last follow up and the number of patients (%) receiving those.

Figure 6.3 Anti-seizure medications tried

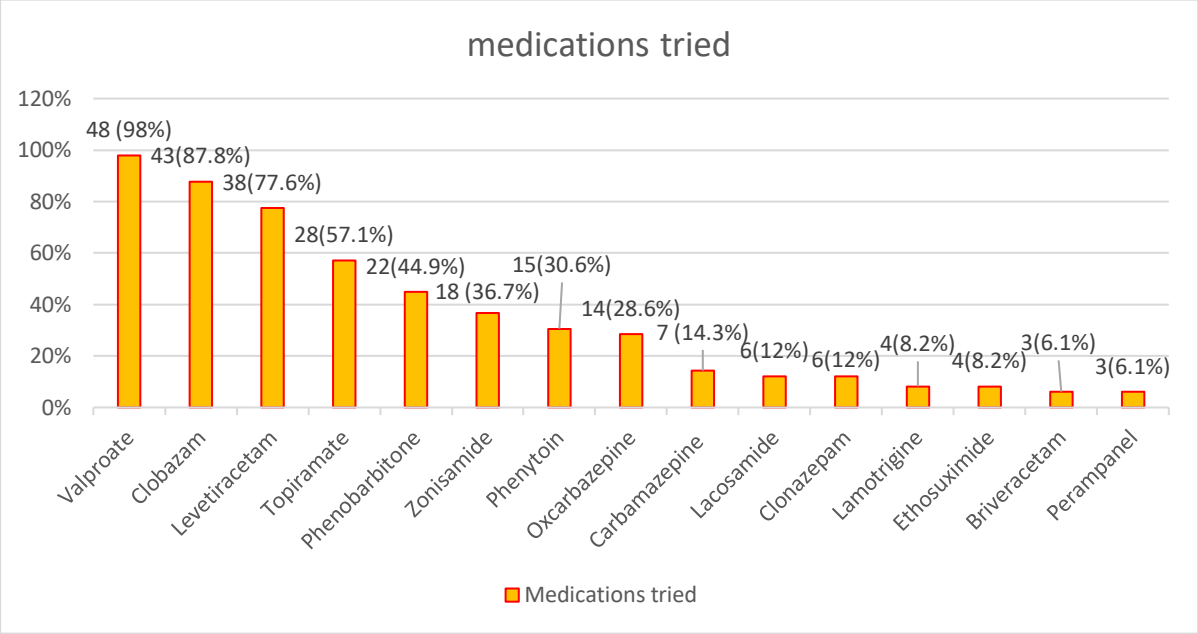


Figure shows the antiseizure medications tried and the number of patients (%) who received those.

Table 6.5 Antiseizure medications data

Polytherapy	41 (85.4%)
Sodium channel blockers	2 (4.2%)
Drug induced worsening Phenytoin Carbamazepine Oxcarbazepine Topiramate Zonisamide Perampanel	9 (18.4%) 2 (4.1%) 2 (4.1%) 2 (4.1%) 1 (2%) 1 (2%) 1 (2%)
Side effects	9 (18.4%)
Allergy (valproate, phenytoin, oxcarbazepine, perampanel)	3 (6.1%)
Hyperammonaemia	5 (10.2 %)

More than 80 % had polytherapy. 9 patients had drug induced worsening of seizures and 9 had side effects like transaminitis, thrombocytopenia, hyperammonemia, allergic reaction and weight gain. Majority had valproate induced side effects, 1 had perampanel induced skin rash.

Table 6.6 Other medications

Pulse steroids	1 (2%)
Pulse steroids + IVIG	1 (2%)
Antipsychotics Risperidone Atomoxetine Methylphenidate Risperidone + Clonidine Clonidine + Aripiprazole	8 (16.7%) 3 (6.1%) 2 (4.1%) 1 (2%) 1 (2%) 1 (2%)
Pyridoxine	6 (12.2%)
Biotin	1 (2%)
Carnitine	20 (40.8%)
Folic acid	2 (4.1%)
Calcium	1 (2%)
Keto diet	15 (31.3%)

(Abbreviations explained in Appendix 3)

Only 31 % could tolerate Keto diet in our cohort.

Table 6.7 Genetic data

Positive genetic test	37 (75.5%)
Genetic test used	
MLPA	7 (14.3%)
CES	37 (75.5%)
WES	5 (10.2%)
SCN1A positivity	29 (59.2%)
Truncating mutations	14 (28.6%)
Missense mutations	15 (30.6%)
ACMG	
Pathogenic	15 (30.6%)
Likely pathogenic	11 (22.4%)
VUS	3 (6.1%)
Other variants	
SCN1B	1 (2%)
GABRA1	1 (2%)
GABARG2	1 (2%)
PCDH19	1(2%)
CHD2	1 (2%)
ALDH7A1 + SCN8A	1 (2%)
GPR98	1 (2%)
CACNA1H	1 (2%)
CACNA1H + SCN1A	1 (2%)
SCN3A + SCN1A	1 (2%)
Zygosity	
Heterozygous	34 (69.3%)
Homozygous	2 (4.1%) (*SCN1A* & *SCN1B*)
Inheritence	
AD	32 (65.3%)
AR	2 (4.1%)
X linked (*PCDH19*)	1 (2%)
Delay in genetic diagnosis (> 2 years)	36 (73.5%)

(Abbreviations explained in Appendix 3)

Seventy five percent had genetic test positivity among which 78 % were *SCN1A* variants and the proportion of missense and truncating variants were similar. Clinical exome sequencing was the most common genetic test administered and majority were pathogenic or likely pathogenic variants. Among the 29 *SCN1A* variants, **23 were novel variants.**

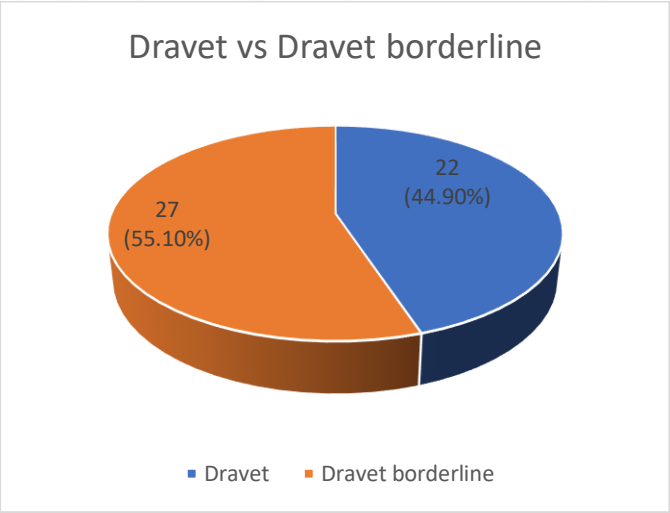
Among the 14 were truncating variants, 8 were frameshift mutations, 2 were deletions, 1 was duplication, 2 were intronic mutations. Among the variants 15 were pathogenic, 11 were likely pathogenic and 3 were promising VUS with phenotype matches. Sanger sequencing done in 1 patient (2%) established denovo variant (c.5339T>C, p.M1780T). One patient with *CACNA1H* VUS was later on confirmed on segregation analysis to be a susceptibility variant in the same gene which was familial with no family history of epilepsy.

Other variants – 8 patients had other variants. 2 patients had other variants along with *SCN1A* – *SCN3A* and *CACNA1H*.

Among the 8, 2 were pathogenic (*CHD2* and *ALDH7A1*). Patient with *ALDH7A1* variant satisfied the clinical criteria for Dravet syndrome, however when genetic test came positive for *ALDH7A1*, she was started on pyridoxine and she responded well and now is seizure free. 2 were likely pathogenic (*PCDH19* and *SCN1B*), rest 4 were VUS.

All the patients had different mutations out of which 30 (81.1 %) were novel variants. More than 70% had a delay in genetic testing for more than 2 years since the onset of illness. One patient underwent genetic testing after 12 years of disease onset.

Figure 6.4 Dravet vs Dravet borderline



After EEG and developmental assessment, only 9 out of 49 had typical Dravet phenotype. Among the Dravet borderline phenotypes, 7 had DB-M phenotype, 12 had DB-SW phenotype, 18 had DB-O phenotype, 3 had DB- GTCS phenotype.

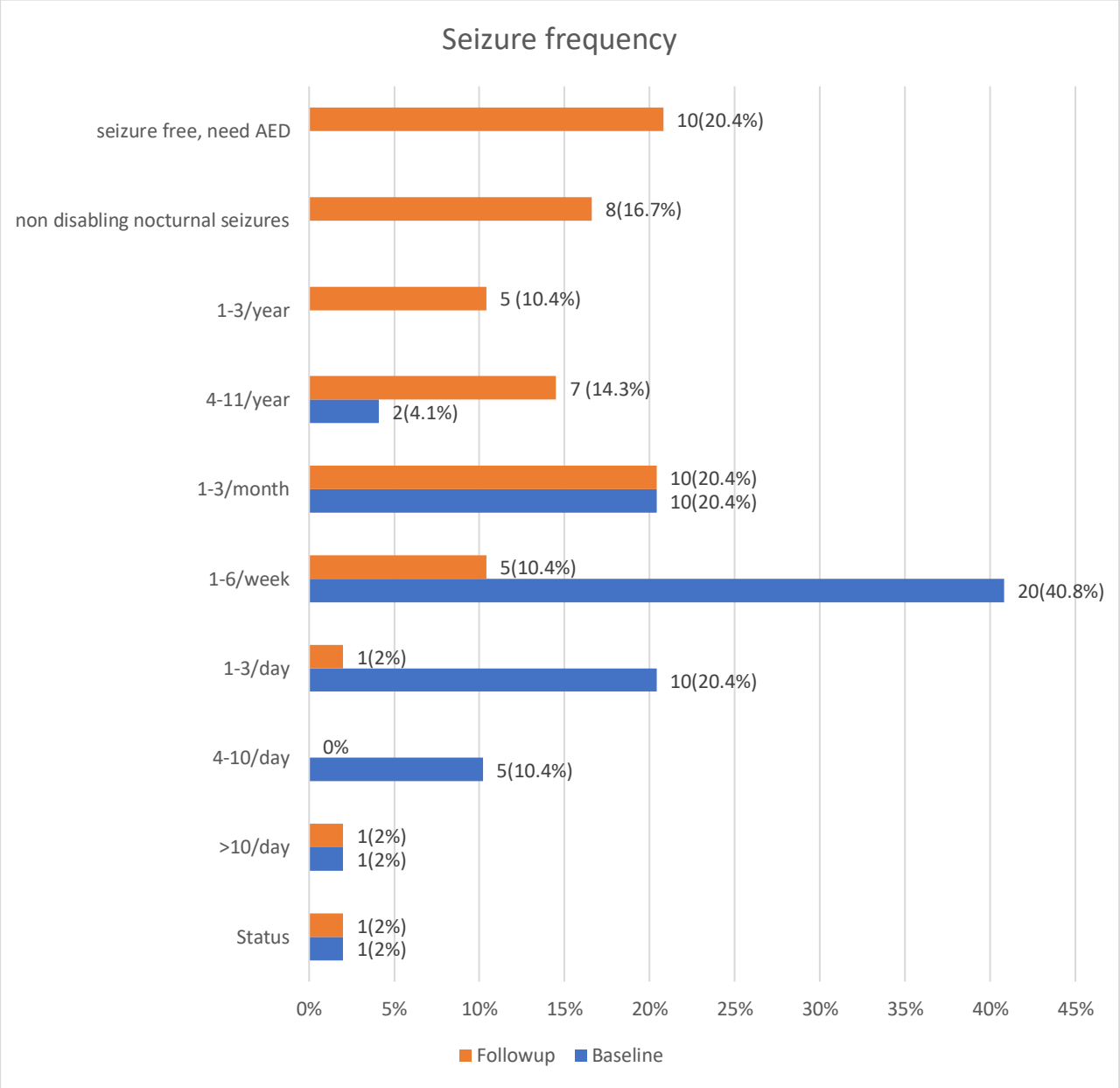
The mean follow up was 3.43 years, ranging from 1 – 17 years.

Table 6.8 Developmental outcome

Language delay	29 (59.2%)
Intellectual disability	21 (42.9 %)
Learning disability	30 (61.2%)
ADHD	22 (44.9 %)
Academic performance	
Good	4 (8.2%)
Fair	17 (34.7%)
Poor	28 (57.1%)
Development	
Age appropriate	16 (32.7%)
Delayed	27 (55.1%)
Developmental quotient	
Mild (>70%)	0
Moderate (30-70%)	14 (28.6%)
Severe (< 30%)	12 (24.5%)
ADL	
Independent	17 (34.7%)
Partly dependent	21 (42.9%)
Fully dependent	10 (20.4%)

(Abbreviations explained in Appendix 3)

Figure 6.5 Seizure score at baseline and on follow up (39)



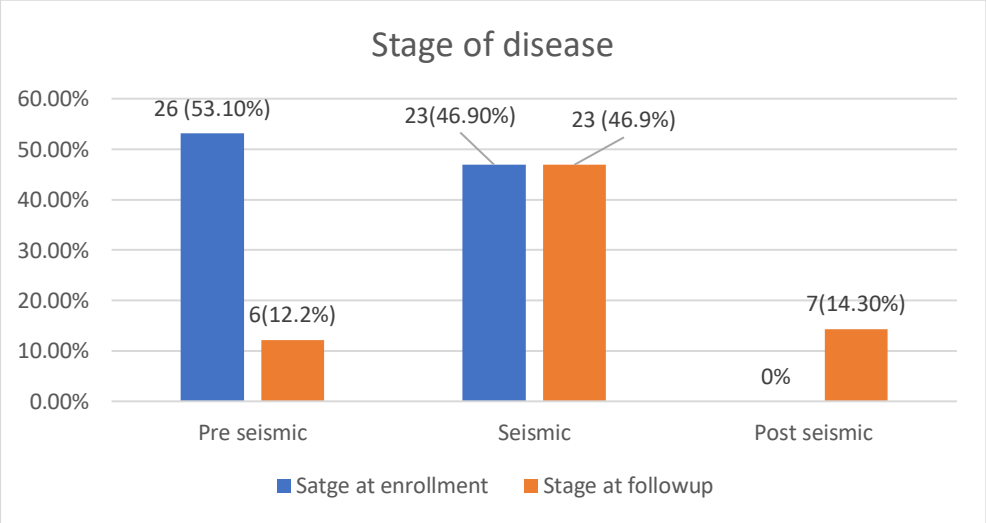
In the figure, seizure score at baseline is depicted in blue and seizure score at followup is depicted in orange. There is a shift towards reduced frequency of seizures on followup.

Table 6.9 Seizure outcome

Seizure score <5 >5	 18 (36.7%) 30 (61.2%)
Seizure freedom	10 (20.4%)
Epilepsy in remission with normal cognition	9 (18.4%)
Epilepsy in remission with developmental delay	9 (18.4%)
Intractable epilepsy with normal development	9 (18.4%)
Intractable epilepsy with developmental delay	21 (42.9%)

One patient died due to respiratory complication at 4 years of age. She had perinatal respiratory distress, developmental delay, febrile seizures since 6 months of age, with clustering and 2 febrile status, unprovoked seizures from 1 year of age, with multiple seizure types and clustering. She was fitting into classical Dravet phenotype, had positive family history of epilepsy, EEG showed multifocal IEDs, photosensitivity with a genetic test positive for *CACNA1H* – VUS variant. No neuroimaging was available for review in this child

Figure 6.6 Stage of disease at baseline and on followup



In the figure, it is seen that the patients were predominantly in the preseismic and seismic stages at the time of enrolment and only 7 patients progressed to the post seismic stage on follow up.

Bivariate analysis

Table 6.10 Comparisons between Dravet vs Dravet borderline phenotypes

	Dravet (22)	**Dravet borderline (27)**	**P value (ANOVA, Fisher's exact)**
Febrile seizures	21 (95.5%)	24 (88.9%)	0.387
Mean age of onset of febrile seizures (SD)	8.4 m ±10.18	5.5 m ± 2.7	0.189
Mean number of febrile status (SD)	5.8 ± 6.4	2.5 ± 2.7	0.135
Mean days of hospitalisation (SD)	10.7 ± 9.5	5.9 ± 3.14	0.127
Clustering of febrile seizures	12 (57%)	19 (76%)	0.149
Mean age of onset of unprovoked seizures (SD)	20.25 ± 26.09	21.07 ±18.3	0.898
Mean number of unprovoked status (SD)	0.50 ± 1.5	0.59 ± 2.04	0.860
Any status	11 (50%)	13 (48%)	0.563
Any clustering	17 (77.3%)	25 (92.6%)	0.133
Family history of febrile seizures	11(50%)	6 (22.2%)	**0.056**
Family history of epilepsy	4 (18.2%)	3 (11%)	**0.056**
Multiple seizure types	22 (100%)	17 (63%)	**0.006**
Vaccine provoked seizures	10 (45.5%)	12 (46.2%)	0.596
Number of ASMs (SD)	3.29 ± 0.9	3.11 ± 0.89	0.506
EEG – multifocal IEDs	6 (30%)	7 (33.3%)	0.543
EEG – generalised IEDs	9 (40.9%)	8 (30.8%)	0.334
BGA slow	9 (40.9%)	12 (44.4%)	0.394
Abnormal MRI	8 (36.4%)	8 (29.6%)	0.835
Developmental age appropriate	7 (33.3%)	9 (33.3%)	0.728
Moderate / severe developmental delay	11 (52.4%)	16 (59.3%)	0.728
ASD	1 (4.5%)	3 (11%)	0.387
ADHD	8 (38%)	14 (51.9%)	0.256
ID	10 (47.6%)	11 (42.3%)	0.472
Language delay	14 (66.7%)	15 (55.6%)	0.315
ADL at followup – independent	9 (42.9%)	8 (29.6%)	0.633
ADL at followup – partly dependent	8 (38%)	13 (48%)	0.633
ADL at followup –	4 (19%)	6 (22.2%)	0.633

completely dependent			
Seizure score >5	15 (71.4%)	15 (55.6%)	0.205
Seizure freedom	3 (13.6%)	7 (25.9%)	0.268
Final outcome – epilepsy in remission with normal cognition	3 (14.3%)	6 (22.2%)	0.437
Final outcome – epilepsy in remission with developmental delay	3 (14.3%)	6 (22.2%)	0.437
Final outcome – intractable epilepsy with normal cognition	6 (28.6%)	3 (11%)	0.437
Final outcome – intractable epilepsy with delayed development	9 (42.9%)	12 (44.4%)	0.437
Positive genetic test	17 (77.2%)	20 (74%)	0.532
SCN1A positive	12 (54.5%)	17 (62.9%)	0.380
Missense mutation	4 (18%)	11 (40.7%)	**0.099**
Truncating mutation	8 (36.3%)	6 (22.2%)	**0.099**

After EEG and developmental assessment, only 9 fulfilled the criteria for typical Dravet and 40 were Dravet borderline phenotype. Among the Dravet borderline phenotypes, 7 had DB-M phenotype, 12 had DB-SW phenotype, 18 had DB-O phenotype, 3 had DB- GTCS phenotype.

On subgroup analysis of these (9 typical Dravet vs 40 Dravet borderline), febrile clonic seizures were found to be significantly associated with typical Dravet (p- 0.002) compared to Dravet borderline phenotypes. Unprovoked tonic seizures were significantly more common in Dravet borderline group (p- 0.031). A non significant propensity was seen among typical Dravet phenotype towards febrile status, family history of febrile seizures or epilepsy and truncating variants whereas Dravet borderline phenotype had propensity towards clustering of seizures and missense *SCN1A* variants. MRI abnormalities were similar among Dravet and Dravet borderline groups.

Among Dravet group, 2 had worsening with oxcarbazepine, 1 each had worsening with topiramate, zonisamide, phenytoin and perampanel. Among Dravet borderline group, 2 had worsening with carbamazepine and 1 had with phenytoin.Though clinical phenotypes showed 55.1 % Dravet borderline and 44.9% typical Dravet phenotype, after EEG and developmental assessment, only 9 out of 49 had typical Dravet phenotype. Among typical Dravet, 6 out of

10 (or is this 9) had *SCN1A* mutations, 83.3% were truncating mutation and 16.7% were missense mutation. In case of DB-M, 6 out of 7 had *SCN1A* mutations, 66.7% were missense and 33.3% were truncating mutation. In case of DB SW, 7 out of 12 had *SCN1A* mutations, 4 had missense, 2 had truncating deletion and 1 had truncating duplication mutation. Among DB-O, 10 out of 18 had *SCN1A* mutations, 46.2% were missense and 30.8% were truncating mutation. None of the DB GTCS variants had *SCN1A* mutation, 1 had *GABRG2* mutation, 1 had *PCDH19* mutation and 1 had no mutation.

Table 6.11 Genetic test positive vs negative test group comparisons

	Positive genetic test (37)	**Negative genetic test (12)**	**P value (ANOVA, Fisher's exact)**
Febrile seizures	34 (91.9%)	11 (91.7%)	0.688
Mean age of onset of febrile seizures (SD)	6.4 m ± 6.5	8.2 m ± 9.4	0.468
Mean number of febrile status (SD)	4.3 ± 5.4	2.7 ±1.7	0.569
Mean days of hospitalisation (SD)	8.6 ± 7.5	6 ± 4.6	0.520
Clustering of febrile seizures	22 (59.4%)	9 (75%)	0.215
Mean age of onset of unprovoked seizures (SD)	19.6 m ± 23.3	24 m ±17.4	0.555
Mean number of unprovoked status (SD)	0.7 ± 2.05	0	0.227
Any status	20 (54.1%)	4 (33.3%)	0.180
Any clustering	31 (83.8%)	11 (91.7%)	0.445
Dravet phenotype	17 (45.9%)	5 (41.7%)	0.532
Dravet borderline phenotype	20 (54.1%)	7 (58.3%)	0.532
Multiple seizure types	29 (78.3%)	10 (83.3%)	0.137
Vaccine provoked seizures	16 (44.4%)	6 (50%)	0.498
Positive family history	17 (45.9%)	7 (58.3%)	0.340
Number of ASMs (SD)	3.19 ± 0.9	3.17 ± 0.8	0.927
EEG – multifocal IEDs	11 (29.7%)	2 (16.6%)	0.308
EEG – generalised IEDs	13 (35.1%)	4 (33.3%)	0.576
BGA slow	16 (43.2%)	5 (41.7%)	0.814
Abnormal MRI	11 (29.7%)	5 (41.7%)	0.499
Developmental age appropriate	13 (36.1%)	3 (25%)	0.623

Moderate / severe developmental delay	20 (55.6%)	7 (58.3%)	0.623
ASD	3 (8.1%)	1 (8.3%)	0.688
ADHD	15 (41.7%)	7 (58.3%)	0.251
ID	16 (45.7%)	5 (41.7%)	0.539
Language delay	22 (61.1%)	7 (58.3%)	0.563
ADL at followup – independent	12 (33.3%)	5 (41.7%)	0.468
ADL at followup – partly dependent	15 (41.7%)	6 (50%)	0.468
ADL at followup – completely dependent	9 (25%)	1 (8.3%)	0.468
Seizure score >5	23 (63.9%)	7 (58.3%)	0.494
Seizure freedom	6 (16.2%)	4 (33.3%)	0.202
Final outcome – epilepsy in remission with normal cognition	6 (16.7%)	3 (25%)	0.777
Final outcome – epilepsy in remission with developmental delay	7 (19.4%)	2 (16.7%)	0.777
Final outcome – intractable epilepsy with normal cognition	6 (16.7%)	3 (25%)	0.777
Final outcome – intractable epilepsy with delayed development	17 (47.2%)	4 (33.3%)	0.777

No significant differences were noted among patients who tested positive and negative for variants following genetic testing. All 3 patients who had facial dysmorphism and all 5 patients who had neurocutaneous markers had positive genetic test.

Eleven out of genetic test positive group and 5 out of genetic test negative group had MRI abnormalities. The abnormalities included focal or diffuse atrophy, white matter hyperintensity, corpus callosal atrophy, malformations and periventricular leukomalacia.

36.1 % of genetic test positive group had developmental delay at baseline whereas 55.6% had developmental delay on followup.

Seven out of genetic test positive group and one out of genetic test negative group were taking medications for hyperactivity.

Figure 6.7 Dravet borderline phenotypes

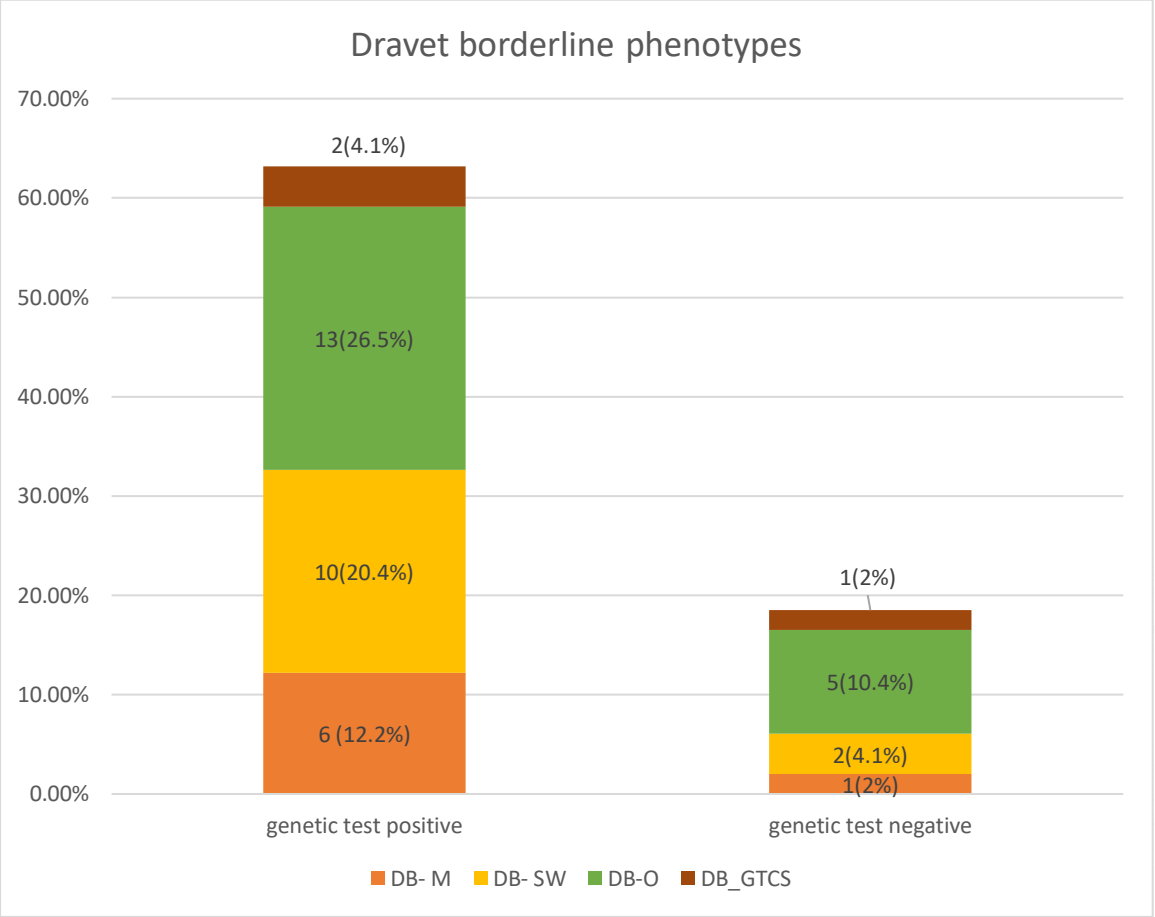


Figure showing the distribution of various Dravet borderline phenotypes among the genetic test positive and negative group. DB-O predominated in both the groups followed by DB- SW.

Table 6.12 Missense vs Truncating variants group comparisons among *SCN1A* positive subgroup

	Missense (15)	**Truncating (14)**	**P value (ANOVA, Fisher's exact)**
Febrile seizures	13 (86.6%)	14 (100%)	0.259
Mean age of onset of febrile seizures (SD)	5.3 m ± 2.2	4.6 m ± 2.7	0.530
Mean number of febrile status (SD)	3.2 ± 3.3	2.8 ±4.0	0.823
Mean days of hospitalisation (SD)	9.7 ± 9.3	6.8 ± 5.2	0.547
Clustering of febrile seizures	10 (71.4%)	8 (57%)	0.347
Mean age of onset of unprovoked seizures (SD)	23.5 m ± 20.6	16.8 m ± 30.3	0.492
Mean number of unprovoked status (SD)	0.2 ± 0.4	1.14 ± 2.7	0.203
Any status	9 (60%)	6 (42.9%)	0.291
Any clustering	13 (86.7%)	11 (78.6%)	0.465
Dravet phenotype	4 (26.7%)	8 (57.1%)	**0.099**
Dravet borderline phenotype	11 (73.3%)	6 (42.9%)	**0.099**
Positive family history	5 (33.3%)	4 (28.6%)	0.550
Multiple seizure types	9 (64.3%)	13 (92.9%)	**0.099**
Vaccine provoked seizures	9 (60%)	7 (53.8%)	0.521
Number of ASMs (SD)	3.07 ± 0.96	3.43 ± 0.514	0.222
EEG – multifocal IEDs	4 (33.3%)	5 (38.5%)	0.560
EEG – generalised IEDs	5 (35.7%)	7 (50%)	0.352
BGA slow	7 (46.7%)	6 (42.9%)	0.920
Abnormal MRI	5 (33.3%)	3 (21.4%)	0.772
Developmental age appropriate	5 (35.7%)	5 (41.7%)	0.536
Moderate / severe developmental delay	9 (64.3%)	7 (58.3%)	0.536
ASD	1 (6.7%)	2 (14.3%)	0.473
ADHD	6 (40%)	7 (50%)	0.434
ID	8 (53.3%)	6 (42.9%)	0.424
Language delay	9 (60%)	9 (64.3%)	0.558
ADL at followup – independent	4 (26.7%)	5 (35.7%)	0.689

ADL at followup – partly dependent	7 (46.7%)	7 (50%)	0.689
ADL at followup – completely dependent	4 (26.7%)	2 (14.3%)	0.689
Seizure score >5	9 (60%)	10 (71.4%)	0.400
Seizure freedom	4 (26.6%)	0	**0.057**
Final outcome – epilepsy in remission with normal cognition	2 (13.3%)	2 (14.3%)	0.842
Final outcome – epilepsy in remission with developmental delay	4 (26.7%)	2 (14.3%))	0.842
Final outcome – intractable epilepsy with normal cognition	2 (13.3%)	3 (21.4%)	0.842
Final outcome – intractable epilepsy with delayed development	7 (46.7%)	7 (50%)	0.842

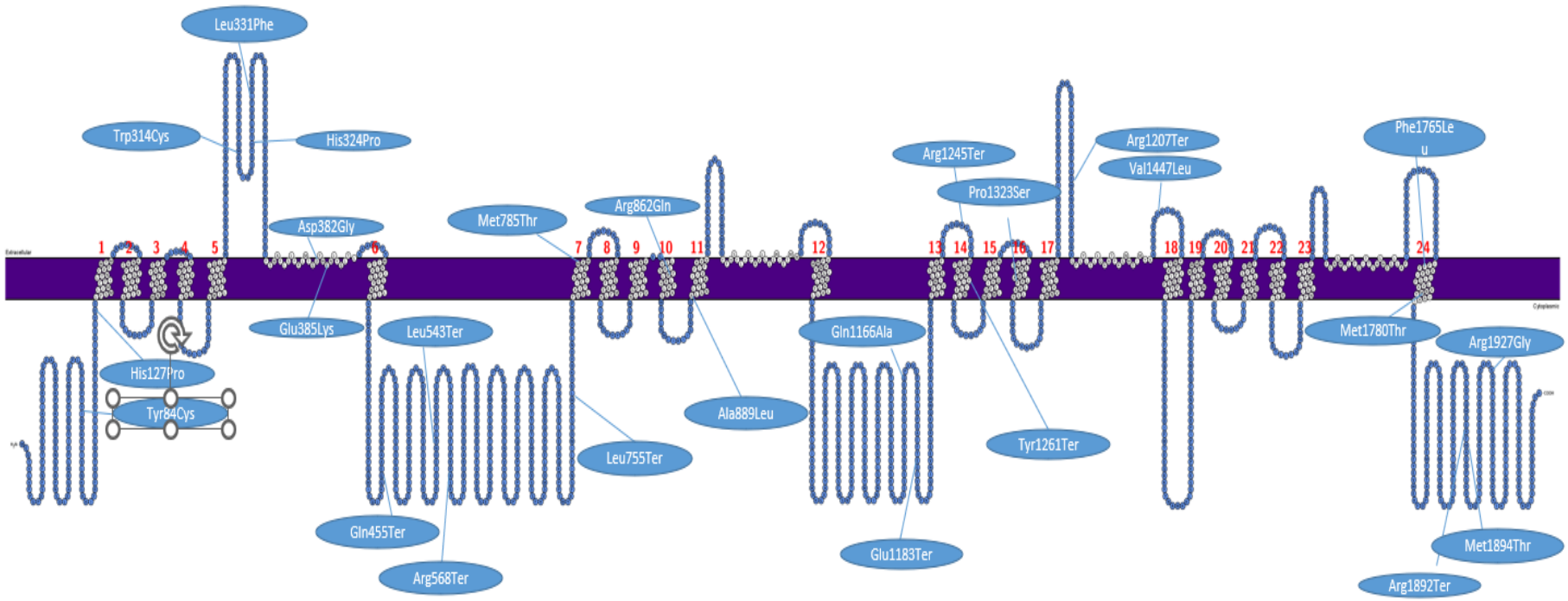
46.7 % of missense mutations had slow background activity in EEG compared to 42.9% of truncating mutations. 7.1% of truncating mutations had photosensitive myoclonus. 26.7% of missense mutations and 14.3% of truncating mutations had marked activation of IEDs in sleep, 53.3% of missense mutations had continued activation in sleep compared to 78.6% of truncating mutations. Sleep was normal in 6.7 % of missense mutations and none in truncating mutations. 20% of missense mutations and 21.4% of truncating mutations had slow for age posterior dominant rhythm.13.3% of missense mutations had poorly formed sleep architecture and 6.7 % had absent sleep architecture whereas it was well formed in all truncating mutations.

The 1 patient who had consanguinity had truncating mutation. Among the 2 patients who had sibling affliction, 1 had missense mutation and 1 had truncating mutation. 50% of truncating mutations and 28.6% of missense mutations had baseline developmental delay. 21.4 % of missense mutations had truncal ataxia whereas 14.3% of truncating mutations had truncal and appendicular ataxia.

Among missense mutations, 1 patient had worsening with phenytoin,2 with oxcarbazepine, 1 with perampanel. Among truncating mutations, 1 had worsening with carbamazepine and 1 had with topiramate.

A non significant propensity for typical DS was noted among children with truncating variants while DB phenotypes were more prevalent among children with missense variants. Multiple seizure types were also apparent among children with truncating variants and seizure freedom was more likely among children with missense variants although the differences were not statistically significant (p - 0.099) No significant differences were noted for other clinical or electrophysiological variables as listed.

Figure 6.8 – Location of the identified variants within the *SCN1A* protein



Schematic diagram showing the alpha subunit of voltage gated sodium ion channel protein encoded by *SCN1A* gene and the domain organization as well as positions of amino acid changes have been marked. Alpha subunit has 4 homologous domains with each having 6 transmembrane segments (labelled 1-24, D1: 1-6, D2 : 7-12, D3: 13-18, D4: 19-24). **Ref: Protter – open source tool for visualization of proteoforms and interactive integration of annotated and predicted sequence features**

Table 6.13 Comparison between patients with seizure score < 5 vs ≥ 5

	Seizure score <5 (18)	Seizure score ≥5 (30)	P value (ANOVA, Fisher's exact)* significant
Febrile seizures	16 (84.2%)	29 (96.7%)	0.155
Mean age of onset of febrile seizures (SD)	7.5 m ± 8.4	6.5 m ± 6.9	0.696
Mean number of febrile status (SD)	2.25 ± 1.8	4.6 ± 5.5	0.409
Mean days of hospitalisation (SD)	11.5 ± 1.9	7.07 ± 7.8	0.288
Clustering of febrile seizures	10 (58.8%)	21 (72.4%)	0.265
Mean age of onset of unprovoked seizures (SD)	15.1 m ±10.6	24.3 ± 26.3	0.166
Mean number of unprovoked status (SD)	0.39 ± 0.9	0.67 ± 2.1	0.614
Any status	7 (36.8%)	17 (56.7%)	0.145
Any clustering	16 (84.2%)	26 (86.7%)	0.561
Dravet phenotype	7 (36.8%)	15 (50%)	0.273
Dravet borderline phenotype	12 (63.2%)	15 (50%)	0.273
Multiple seizure types	12 (66.6%)	26 (86.6%)	0.109
Vaccine provoked seizures	6 (31.6%)	16 (55.2%)	**0.095**
Positive family history	9 (47.3%)	15 (50%)	0.858
Number of ASMs (SD)	2.8 ± 1.1	3.37 ± 0.6	**0.072**
EEG – multifocal IEDs	3 (21.4%)	10 (37%)	0.257
EEG – generalised IEDs	4 (21%)	13 (44.8%)	**0.083**
BGA slow	7 (36.8%)	14 (46.7%)	0.683
Abnormal MRI	6 (31.6%)	10 (33.3%)	0.591
Developmental- age appropriate	9 (50%)	7 (23.3%)	**0.046***
Moderate / severe developmental delay	6 (33.3%)	21 (70%)	**0.046***
ASD	1 (5.3%)	3 (10%)	0.493
ADHD	8 (44.4%)	14 (46.7%)	0.560
ID	4 (23.5%)	17 (56.7%)	**0.028 ***
Language delay	9 (50%)	20 (66.7%)	0.201
ADL at followup – independent	9 (50%)	8 (26.6%)	0.179
ADL at followup – partly dependent	5 (27.7%)	16 (53.3%)	0.179
ADL at followup – completely dependent	4 (22.2%)	6 (20%)	0.179

Positive genetic test	13 (72.2%)	23 (76.6%)	0.494
SCN1A positive	10 (55.5%)	19 (63.3%)	0.408
Missense mutation	6 (33.3%)	9 (30%)	0.400
Truncating mutation	4 (22.2%)	10 (33.3%)	0.400

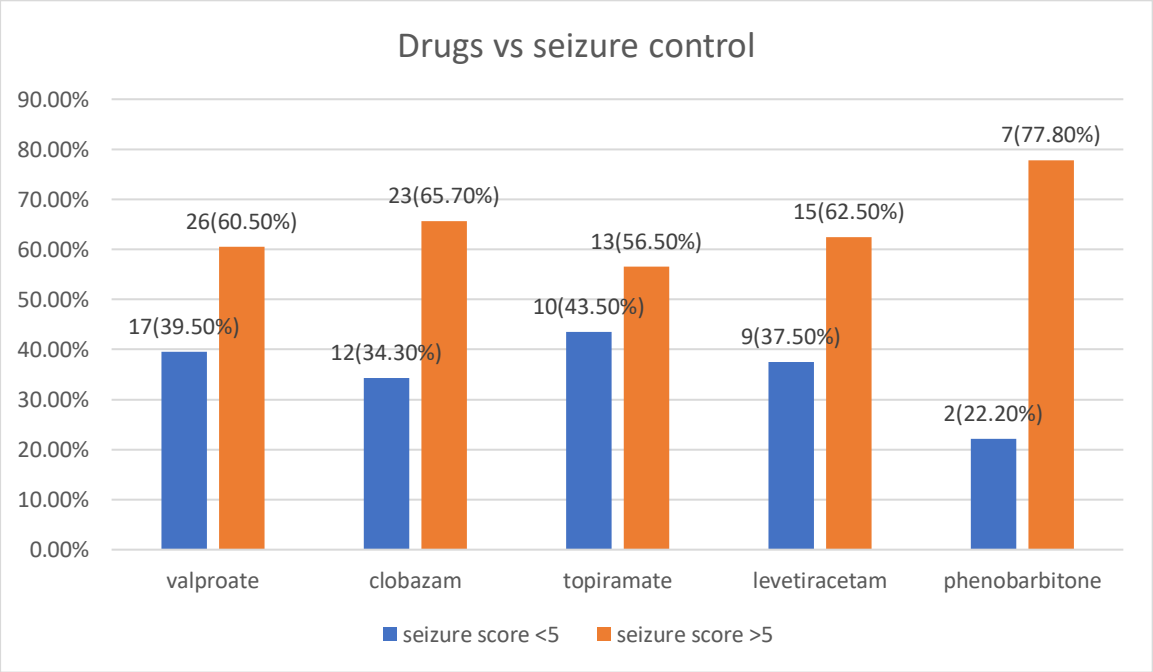
A non significant trend was seen for febrile seizures among the ASM resistant subgroup with seizure score > 5. Those who had a later onset of unprovoked seizures were showing a trend towards seizure score ≥ 5. Any status epilepticus and multiple seizure types were noted among patient group with seizure score ≥5.

Generalised IEDs were more favouring seizure score ≥5. Developmental delay, intellectual disability and learning disability were found to be significantly associated with intractable epilepsy with p values 0.046, 0.028, 0.029 respectively.

Among the 3 pre term deliveries, 2 had intractable epilepsy. Among the 7 patients who had NICU care, 5 had intractable epilepsy. Two children who had pathological jaundice and 1 who had neonatal sepsis had intractable epilepsy.23 % of those with intractable epilepsy had ataxia.

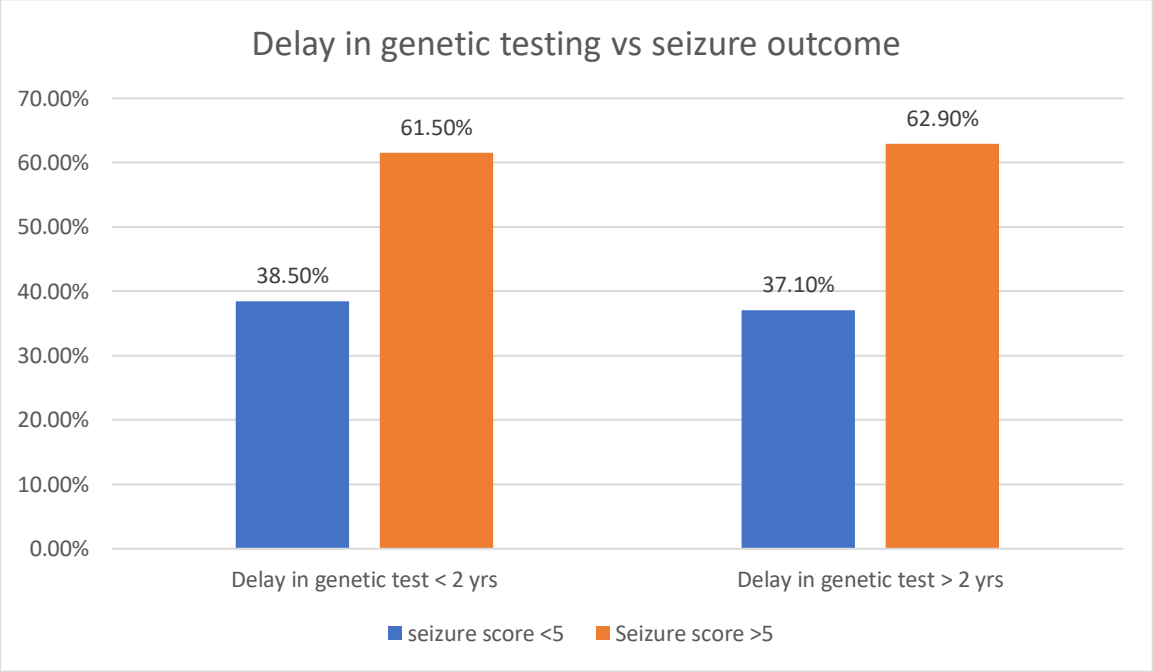
None of the patients with intractable epilepsy had photosensitivity, 1 had poorly formed sleep architecture. Thirty three percent had abnormal MRI.

Figure 6.9 ASM vs seizure control



All 7 patients who were on Zonisamide were having seizure score > 5 which was found to be significant (p- 0.028). Rest of the drugs were not found to have any significant association with seizure control. Two patients who received sodium channel blockers were found to have intractable epilepsy.

Figure 6.10 Delay in genetic testing vs seizure outcome



This was not found to be significant

Table 6.14 Comparison between seizure freedom vs ASM resistant epilepsy

	Seizure free (10)	**ASM resistant epilepsy (38)**	**P value (ANOVA, Fisher's exact)**
Febrile seizures	7 (70%)	37 (97.4%)	**0.025 ***
Mean age of onset of febrile seizures (SD)	11 m ± 11.5	6.1 m ± 6.2	0.108
Mean number of febrile status (SD)	3.5 ± 2.1	4.2 ± 5.3	0.852
Mean days of hospitalisation (SD)	11 ± 1.4	7.6 ± 7.5	0.550
Clustering of febrile seizures	4 (40%)	26 (70.3%)	0.241
Mean age of onset of unprovoked seizures (SD)	19.2 m ±12.1	21.3 m ± 24.2	0.796
Mean number of	0.2 ± 0.4	0.6 ± 2.0	0.485

unprovoked status (SD)			
Any status	4 (40%)	19 (50%)	0.419
Any clustering	9 (90%)	32 (84.2%)	0.546
Dravet phenotype	3 (30%)	18 (47.36%)	0.268
Dravet borderline phenotype	7 (70%)	20 (52.6%)	0.268
Multiple seizure types	6 (60%)	32 (84.2%)	0.143
Positive family history	5 (50%)	18 (47.36%)	0.581
Vaccine provoked seizures	3 (30%)	19 (51.4%)	0.201
Number of ASMs (SD)	2.7 $\pm$ 1.16	3.3 $\pm$ 0.75	**0.051**
EEG – multifocal IEDs	0	12 (36.4%)	**0.064**
EEG – generalised IEDs	3 (30%)	14 (37.8%)	0.474
BGA slow	4 (40%)	17 (44.7%)	0.760
Abnormal MRI	6 (60%)	10 (26.3%)	0.120
Developmental age appropriate	6 (60%)	10 (26.3%)	**0.034***
Moderate / severe developmental delay	2 (20%)	25 (65.8%)	**0.034***
ASD	0	4 (10.5%)	0.379
ADHD	3 (30%)	19 (50%)	0.221
ID	2 (20%)	19 (50%)	0.077
Language delay	3 (30%)	26 (68.4%)	**0.033 ***
ADL at followup – independent	6 (60%)	11 (28.9%)	0.150
ADL at followup – partly dependent	2 (20%)	19 (50%)	0.150
ADL at followup – completely dependent	2 (20%)	8 (21.1%)	0.150
Positive genetic test	6 (60%)	30 (78.9%)	0.202
SCN1A positive	4 (40%)	25 (65.7%)	0.132
Missense mutation	4 (40%)	11 (28.9%)	**0.057**
Truncating mutation	0	14 (36.8%)	**0.057**

Among those with and without seizure freedom, absence of febrile seizures was found to be significantly associated with seizure freedom (p-0.025). Those who had age appropriate development had significant association with seizure freedom. (p-0.022). Language delay and learning disability were significantly associated with ASM resistant epilepsy (p-0.033 and 0.013 respectively), and there was a non significant propensity for intellectual disability and dependency in those with ASM resistant epilepsy.

There was a non significant propensity for early age of onset of febrile seizures and multiple seizure types towards ASM resistant epilepsy. Multifocal IEDs in EEG, *SCN1A* positivity as well as truncating mutations also showed a trend towards ASM resistant epilepsy.

Among the patients who were seizure free on last follow up (N=10), ASM combinations in each of them are listed in the table below:

Table 6.15 – ASM combinations in patients with seizure freedom at last followup (N=10; only 1 of them was following KD)

Patients	ASM combination
Patient 1	Valproate
Patient 2	Valproate + Levetiracetam
Patient 3	Valproate + Clobazam + Levetiracetam + Topiramate + Phenobarbitone
Patient 4	Valproate + Clobazam + Topiramate
Patient 5	Valproate + Clobazam + Levetiracetam
Patient 6	Valproate + Clobazam + Topiramate
Patient 7	Valproate + Levetiracetam + Clonazepam
Patient 8	Valproate + Clobazam + Topiramate
Patient 9	Valproate
Patient 10	Valproate + Clobazam + Topiramate

Table 6.16 – Comparison between moderate / severe developmental delay vs no developmental delay

Two among the 3 pre term deliveries had developmental delay at follow up and 4 among the

	Normal Development (16)	**Developmental delay (27)**	**P value (ANOVA, Fisher's exact)**
Febrile seizures	13 (81.3%)	26 (96.3%)	0.137
Mean age of onset of febrile seizures (SD)	9.8 m ± 12.7	5.5 m ± 2.6	0.104
Mean number of febrile status (SD)	6.5 ± 4.9	4.4 ± 5.5	0.625
Mean days of hospitalisation (SD)	5.5 ± 2.1	8.14 ± 8.1	0.663
Clustering of febrile seizures	6 (46.2%)	20 (74.1%)	**0.085**
Mean age of onset of unprovoked seizures (SD)	15.4 m ± 12.9	19.3 m ± 18.4	0.463
Mean number of unprovoked status (SD)	0.2 ± 0.4	0.8 ± 2.3	0.356
Any status	5 (31.3%)	15 (55.6%)	0.109
Any clustering	14 (87.5%)	23 (85.2%)	0.606
Dravet phenotype	7 (43.8%)	11 (40.7%)	0.548
Dravet borderline phenotype	9 (56.3%)	16 (59.3%)	0.548
Multiple seizure types	12 (75%)	22 (81.4%)	0.846
Vaccine provoked seizures	6 (40%)	15 (55.6%)	0.260
Positive family history	7 (43.8%)	15 (55.6%)	0.247
Number of ASMs (SD)	2.8 ±1.1	3.4 ± 0.69	**0.042 ***
EEG – multifocal IEDs	3 (18%)	8 (29.6 %)	0.424
EEG – generalised IEDs	6 (37.5%)	9 (33.3%)	0.458
BGA slow	4 (25%)	13 (48.1%)	0.422
Abnormal MRI	4 (25%)	8 (29.6%)	0.894
ASD	0	3 (11%)	0.237
ADHD	5 (31.3%)	14 (51.9%)	0.159
Seizure score >5	7 (43.75%)	21 (77.7%)	**0.046 ***
Seizure freedom	6 (37.5%)	2 (7.4%)	**0.034 ***
Positive genetic test	13 (81.25%)	20 (74%)	0.623
SCN1A positive	10 (62.5%)	16 (59.2%)	0.978
Missense mutation	5 (31.25%)	9 (33.3%)	0.536
Truncating mutation	5 (31.25%)	7 (25.9%)	0.536

7 patients who had NICU stay had delayed development. Among those who had delayed development, 1 patient had respiratory distress, 2 had pathological jaundice and 1 had neonatal sepsis.

Developmental delay was significant in those who had febrile status when compared to those without febrile status (P- 0.029) and there was a non significant propensity for any status towards developmental delay.

There was a trend towards developmental delay in patients with febrile seizures and in those with early age of onset of febrile seizures with clustering of febrile seizures.

There was significant association between seizure freedom and development. P – 0.034. Sixty percent of children who had seizure freedom had age appropriate development.

Among 15 patients who had baseline developmental delay, 3 had picked up development at follow up. There was a trend of ADHD more in those with developmental delay.

Three patients who had poorly developed/ absent sleep structures had developmental delay.

Eight among the twelve who had MRI abnormalities had developmental delay.

The mean number of ASM used in those with normal development was 2.8 vs 3.4 in those with delayed development, which is found to be significant (p-0.042) which was expected.

Table 6.17 – Positive family history of febrile seizures / epilepsy versus no family history of seizures or epilepsy

	Positive family history (24)	**Negative family history (25)**	**P value (ANOVA, Fisher's exact) * significant**
Febrile seizures	22 (91.7%)	23 (92%)	0.680
Mean age of onset of febrile seizures (SD)	9.1 m ± 9.7	4.6 m ± 2.1	**0.036***
Mean number of febrile status (SD)	5 ± 6.3	3.1 ± 2.8	0.403
Mean days of hospitalisation (SD)	9 ± 9.2	7.2 ± 4.18	0.582
Clustering of febrile seizures	12 (54.5%)	19 (79.2%)	0.071
Mean age of onset of unprovoked seizures (SD)	20.3 m ± 19.8	21 m ± 24.19	0.904
Mean number of unprovoked status (SD)	0.58 ± 1.6	0.52 ± 2.0	0.904
Any status	12 (50%)	12 (48%)	0.558
Any clustering	19 (79.2%)	23 (92%)	0.192
Dravet phenotype	15 (62.5%)	7 (28%)	**0.016 ***
Dravet borderline phenotype	9 (37.5%)	18 (72%)	**0.016 ***
Multiple seizure types	20 (83.3%)	19 (76%)	0.367
Vaccine provoked seizures	9 (37.5%)	13 (54.2%)	0.193
Number of ASMs (SD)	3.1 ±1.0	3.2 ± 0.77	0.675
EEG – multifocal IEDs	6 (30%)	7 (33.3%)	0.543
EEG – generalised IEDs	7 (29.2%)	10 (41.7%)	0.273
BGA slow	9 (37.5%)	12 (48%)	0.222
Abnormal MRI	8 (33.3%)	8 (32%)	0.800
Developmental age appropriate	10 (41.6%)	13 (52%)	0.247
Moderate / severe developmental delay	14 (58.3%)	12 (48%)	0.247
ASD	2 (8.3%)	2 (8%)	0.680
ADHD	10 (43.5%)	12 (48%)	0.491
ID	7 (31.8%)	14 (56%)	**0.085**
Language delay	14 (60.9%)	15 (60%)	0.593
ADL at followup – independent	9 (39%)	8 (32%)	0.817

ADL at followup – partly dependent	9 (39%)	12 (48%)	0.817
ADL at followup – completely dependent	5 (21.7%)	5 (20%)	0.817
Seizure score >5	15 (65.2%)	15 (60%)	0.471
Seizure freedom	5 (20.8%)	5 (20%)	0.581
Final outcome – epilepsy in remission with normal cognition	5 (21.7%)	4 (16%)	0.756
Final outcome – epilepsy in remission with developmental delay	3 (13%)	6 (24%)	0.756
Final outcome – intractable epilepsy with normal cognition	5 (21.7%)	4 (16%)	0.756
Final outcome – intractable epilepsy with delayed development	10 (43.5%)	11 (44%)	0.756
Positive genetic test	17 (70.8%)	20 (80%)	0.340
SCN1A positive	9 (37.5%)	20 (80%)	0.550
Missense mutation	5 (20.8%)	10 (40%)	0.550
Truncating mutation	4 (16.6%)	10 (40%)	0.550

The 1 patient who had history of consanguinity did not have a positive family history.

Those who had positive family history had a later onset of febrile seizures than those with negative family history. Clustering of seizures were more seen in those without family history.

Patients with family history had a significant propensity towards typical Dravet phenotype.

There was no significant difference between the 2 groups with respect to type of seizures or the medications used except for unprovoked clonic seizures which were more common in those with negative family history (p-0.040) and myoclonic jerks were more common in those with positive family history (p-0.004) as expected, as Dravet phenotype were more common in those with positive family history. There was a non significant trend towards intellectual disability in those without family history.

There were no significant differences in EEG abnormalities or MRI substrates between the 2 groups.

Multivariate analysis was attempted with variables which had p value < 0.1 in bivariate analysis of seizure freedom vs ASM resistant epilepsy so as to determine the independent predictors of seizure freedom. However, coherent modules could not be generated possibly due to low sample size.

Table 6.18 Multivariate analysis of developmental delay predictors

Variables	OR	95% CI
Age at onset of febrile seizures	0.978	0.865-1.105
Clustering of febrile seizures	0.272	0.048-1.534
Any Status epilepticus	0.188	0.029-1.204
Number of antiseizure medications	1.25	0.450-3.467
Seizure freedom	0.078	0.005-1.161

On multivariate analysis, age at onset of febrile seizures, clustering of febrile seizures, status epilepticus, number of antiseizure medications, seizure freedom were not found to be significantly associated with developmental delay.

Discussion

Our study is the first of its kind to emerge from the Indian subcontinent in a large cohort of patients with this rare DEE phenotype with around 60% of patients having tested positive for *SCN1A* variants. Major findings include: a) the higher prevalence of truncating mutations among typical DS and ASM resistant epilepsy, b) moderate – severe developmental delay and language delay in patients with ASM resistant epilepsy, c) later onset of febrile seizures and higher prevalence of typical DS in those with positive family history of febrile seizures or epilepsy, d) higher prevalence of polypharmacy and clustering of febrile seizures in those with moderate to severe developmental delay and e) higher prevalence of seizure freedom among those who had no febrile seizures.

Predictors of ASM resistant epilepsy included presence of febrile seizures, truncating variants and multifocal IEDs in EEG. Developmental delay appeared to be related to clustering of febrile seizures and polypharmacy. However, none of the variables were found to be significant on multivariate analysis of predictors of developmental delay. Coherent modules could not be generated for multivariate analysis on predictors of seizure freedom. A positive genetic test did not seem to have an impact on seizure or developmental outcomes in our cohort in which patients were rigorously classified as either DS or DB phenotypes suggesting that the clinical phenotype is more relevant in this DEE and genetic testing has therapeutic implications with regard to ASM selection. Variant calling may also have a role in prognostication with regard to seizure outcomes as demonstrated by the trends noted on comparisons between truncating and missense variants.

Our study identified *SCN1A* mutations in nearly 3/5 th of the patients diagnosed with Dravet syndrome or Dravet borderline phenotype during the time period 2015-2022. Challenges encountered were delay in genetic diagnosis, due to lack of affordable testing and inability to test in trios throughout the cohort to establish de novo variants. Hence interpretation of VUS were largely confined to literature search and in silico prediction models such as SIFT, PolyPhen and Mutation transfer with significance attached if all 3 predicted damaging effects.

There was no gender predominance in our cohort, a result which is consistently observed in other studies like Gertler et al (40), Nabbout et al (41) Korff et al (8). There was no gender difference when compared between Dravet and Dravet borderline phenotype unlike what the study by in Ohmori et al where females suffered more from typical Dravet and males suffered more from Dravet borderline phenotype. (42)

Almost equal proportion of missense and truncating variants as noted in previous studies by Korff et al (8), Zuberi et al (43). Truncating variants were more prevalent in studies like Jiang et al, (25) Ishii et al (44) and missense variants were more prevalent in studies like Kanai et al (9)

In our study, all the patients had different mutations out of which 81.1 % were novel variants similar to the study by Harkin et al which indicates the mutational heterogeneity which is very characteristic of *SCN1A*. (3) In the same study by Harkin et al, 96% of those who had available parental DNA had de novo mutations. In our study, there was only 1 patient whose parental DNA was available, as we could not attempt uniform segregation analysis throughout the cohort. The other variants which were identified which may have therapeutic implications were *SCN1B, GABRA1, GABRG2, PCDH19, SCN3A, CHD2, CACNA1H.*Two patients had *CACNA1H* (one established as familial) and 1 each had the rest of the mutations. Two among them had additional variants along with *SCN1A – SCN3A* and *CACNA1H*. Among the total study population, 73.5% had delay in genetic testing for more than 2 years. *PCDH 19* mimic Dravet syndrome clinically but the degree of cognitive impairment is usually mild to moderate. (45)

No recurring variants were identified in our cohort and 81.1 % were novel. This needs to be considered from the perspective of the high prevalence of *SCN1A* pathogenic /likely pathogenic variants in other larger databases in published literature. The various epilepsy syndromes associated with *SCN1A* variants apart from DS are ICEGTC, Lennox Gastaut syndrome, Myoclonic astatic epilepsy/ Doose syndrome, West syndrome, cryptogenic focal epilepsy, cryptogenic generalised epilepsy, infantile spasms, Rasmussen's encephalitis, severe idiopathic generalised epilepsy of infancy and epilepsy of infancy with migrating focal seizures. (46) Hence a rigorous variant interpretation protocol is mandatory prior to attaching the clinical relevance of an identified *SCN1A* variant.

Table 7.1 *SCN1A* variants and location (* novel variants)

	Location	Gene variant	Aminoacid change
Patient 1	Exon 22	c.3783C>G	p.Tyr1261Ter*
Patient 2	Exon 13	c1702 C>T	p.Arg568Ter*
Patient 3	Exon 22	c.3733C>T	p.Arg1245Ter*
Patient 4	Exon 29	c.5674C>T	p.Arg1892Ter*
Patient 5	Exon 15	c.2665delG	p.Ala889LeufsTer5*
Patient 6	Exon 12	c.1628T>A	p.Leu543Ter*
Patient 7	**Intron 4**	chr2:166911147C>T c.602+1G>A	
Patient 8	**Intron 3**	c.264+2T>G	
Patient 9	Exon 13	c.2264T>G	p.Leu755Ter*
Patient 10	Exon 17	chr2:166872170-166872171delTG	pGln1166AlafsTer6*
Patient 11	Exon 9	c.1363C>T	p.Gln455Ter*
Patient 12	Exon 7	c.992dup	p.Leu331PhefsTer9
Patient 13	**Intron 20**	c.4002+2T>C	
Patient 14	Exon 24	c.4219C>T	p.Arg1407Ter*
Patient 15	Exon 27	c.5339T>C	p.M1780T
Patient 16	Exon 26	c.5293T>C	p.Phe1765Leu*
Patient 17	Exon 6	c.942G>C	p.Trp314Cys*
Patient 18	Exon 14	c.2585G>A	p.Arg862Gln
Patient 19	Exon 20	c.3967C>T	p.Pro1323Ser*
Patient 20	Exon 26	c.5681T>C	p.Met1894Thr*
Patient 21	Exon 2	c.380A>C	p.His127Pro*
Patient 22	Exon 1	c.251A>G	p.Tyr84Cys
Patient 23	Exon 13	c.2354T>C	p.Met785Thr
Patient 24	Exon 20	c.3547G>T	p.Glu1183Ter*
Patient 25	Exon 7	c.971A>C	p.His324Pro*
Patient 26	Exon 8	c1145A>G	p.Asp382Gly*
Patient 27	Exon 28	c.5779A>G	p.Arg1927Gly*
Patient 28	Exon 23	chr2:166854685C>A c.4339G>T	p.Val1447Leu*
Patient 29	Exon 8	c.1153G>A	p.Glu385Lys*

Fs- frame shift ; Ter – terminating stop codon

Missense mutations can abolish channel function, possibly by altering the channel properties, trafficking, subcellular localization or interactions with other molecules. (11) Three intronic variants were also noted in our cohort, all were pathogenic and these were known to lead to abnormal spilicing.

Dravet vs Dravet borderline phenotype

23 out of 40 (57.5%) Dravet borderline phenotypes had *SCN1A* mutations, higher than 26% reported by Fukuma et al.(47), lower than 88% by Ohmori et al. (42) In the study by Harkin et al, 69% of borderline phenotypes had *SCN1A* mutations. (3) 66.7% of Dravet, 85.7% of DB-M, 83.3% of DB SW and 72.2% of DB-O had positive genetic test. We found that typical Dravet phenotypes had a non significant trend of positive family history and towards truncating variants as opposed to the study by Harkin et al, where the proportion of missense vs truncating mutations is similar in both typical Dravet as well as Dravet borderline phenotypes. (3) In the study by Ohmori et al, *SCN1A* mutations were found in 72.7% of typical Dravet and 88.2% of borderline phenotypes. (42) In the study by Harkin et al, 52% of borderline phenotypes had missense mutations, 40 % had truncating mutations and the rest were splice site mutations.

On comparing clinical phenotypes, typical Dravet and Dravet borderline, there was no significant difference between clinical features, age of onset of febrile or afebrile seizures, clustering of seizures or status, vaccine provoked seizures, multiple seizure types, type of seizure, except for myoclonic jerks the absence of which was considered as the inclusion criteria for DB-M, sleep activation of seizures, EEG or MRI characteristics, need for polytherapy, the final developmental or neurocognitive outcome as well as control of seizures.

Family history of febrile seizures was found to be seen more with typical Dravet when compared to Dravet borderline. A non significant propensity was seen among Dravet phenotype towards febrile status and truncating mutations whereas Dravet borderline phenotype had propensity towards clustering of seizures and missense *SCN1A* variants.

Family history of febrile seizures / epilepsy vs no family history

Patients without family history of febrile seizures/ epilepsy had an earlier age of onset of febrile seizures than those with positive family history. Clustering of seizures were more seen

in those without family history. Typical Dravet phenotype was more seen in patients with family history and Dravet borderline phenotypes were more in those without family history. (p-0.016) There was no significant difference between the 2 groups with respect to presence of baseline development, febrile seizures, type of seizures or the medications used except for unprovoked clonic seizures which were more common in those with negative family history (p-0.040) and myoclonic jerks were more common in those with positive family history (p-0.004) as expected, as Dravet phenotype were more common in those with positive family history. There was a non-significant trend towards intellectual disability in those without family history. There were no significant differences in the EEG or MRI characteristics, neurodevelopmental or seizure outcome between the 2 groups.

As per literature, 15-35% of Dravet syndrome patients can have family history of febrile seizures or epilepsy. The most common phenotype observed is GEFS+ in first degree relatives. (48) In the study by Depienne et al, they demonstrated 2 children of unaffected parents manifesting deleterious *SCN1A* mutations in 2 unrelated families and they could identify somatic and germline mosaicism in the parents. (29) Similar reports of affected children with inherited *SCN1A* variant from asymptomatic or mildly symptomatic parents were also reported by Annesi et al. in 2003 (49), Gennaro et al. in 2003 (50), Nabbout et al. in 2003 (41), Fukuma et al. in 2004 (47) and Kimura et al. in 2005 (51) . This may suggest the importance of segregation analysis and determination of germline mosaicism. None of our patients had a first degree relative diagnosed with DS, however 17 patients had a positive family history of febrile seizures. In the absence of segregation analysis, parental mosaicism cannot be commented upon. It may also be due to the variable penetrance leading to variable expressivity and consequent phenotypic heterogeneity. (52)

Genetic test positive vs negative

There were no significant differences in the clinical phenotypes, EEG or MRI parameters or neurocognitive outcome with respect to genetic test positivity, thereby emphasizing the relevance of an electroclinical syndromic diagnosis and exclusion of mimic etiologies such as structural or metabolic although a sizeable proportion of patients may not have any etiology confirmed despite MRI and genetic testing. The fact that one patient who met the criteria for DS was diagnosed to have pyridoxine dependency with an incidental *SCN8A* variant also suggests the importance of genetic testing to confirm the diagnosis and exclude alternative etiologies. A non significant trend was seen in genetic test negative patients towards multiple

seizure types suggesting the possibility that non *SCN1A* genes could be responsible for similar phenotypes as has been noted with *SCN1B, HCN1, GABRG2, GABRA1, KCNA2.*(26,48,53,54)

Valproate, clobazam, levetiracetam and topiramate were the commonly used drugs in our cohort. Valproate was prescribed for 89.6% of the total population, Clobazam for 72.9%, levetiracetam for 50% and topiramate for 47.9%. There were no significant differences between the drug used and final neurodevelopmental outcome or seizure control except for Zonisamide, where all the 7 patients who were on Zonisamide had seizure score >5. In the study by Shi XY et al, Zonisamide had 36% efficacy in *SCN1A* positive DS as opposed to our findings. (55) Medications found to be beneficial in our study included combinations of valproate with topiramate, levetiracetam, phenobarbitone and clobazam among the various combinations tried. This is reflective of the current recommendations for standard ASM practice guidelines in DS. (56) Non availability of newer 3rd generation ASM in India, such as stiripentol, fenfluramine and cannabidiol also potentially impacted the ASM benefits and outcomes in our cohort. However, in the absence of head to head studies comparing these newer molecules with standard ASM, the actual impact of this needs to be studied.

Missense vs truncating mutations

A non-significant propensity for typical DS was noted among children with truncating variants while DB phenotypes were more prevalent among children with missense variants. Multiple seizure types were also apparent among children with truncating variants and seizure freedom was more likely among children with missense variants although the differences were not statistically significant. No significant differences were noted for other clinical (including seizure or developmental outcomes) or electrophysiological variables. This represents a novel finding in our study.

Our results however indicated a trend towards ASM resistant epilepsy, multiple seizure types and typical DS among patients with truncating mutations. This can be attributed to potentially greater loss of function effects on the sodium ion activated GABAergic interneurons leading to lesser inhibition on EPSP along pyramidal cells (57). However, given the absence of significant differences on seizure scores or developmental outcomes, missense variants can also lead to significant effects in terms of epilepsy and developmental outcomes suggesting that missense variant-related *SCN1A*-mediated epilepsy in our cohort also predicts potential evolution as DEE. Irrespective of the nature of the variant *SCN1A* mediated epilepsy

clinically meeting criteria for DS or DB phenotypes represents a prototype DEE. In our study we could not find any significant differences between missense and truncating mutations with respect to seizure freedom or developmental delay, EEG or MRI abnormality, treatment or prognosis. In the study by Zuberi et al, there were also no significant differences in the types of seizures among missense or truncating mutations, though the age of onset of seizures were earlier in truncating mutations with respect to myoclonic, atypical absence and prolonged seizures. However no differences in developmental outcome were seen between the two groups. (43) In the study by Gertler et al, out of 137 patients, 96% had positive genetic test, out of which 36% were missense mutations and 54% were truncating mutations. Seizure onset, type of seizures, status epilepticus, neurocognitive outcome as well as pharmacoresponsiveness were not found to be significantly different between the two groups. (40) In the study by Ishii et al, they could not find any significant difference between the age of onset among missense and truncating mutations. (44)

Seizure score <5 vs >5

Generalised IEDs were more favouring seizure score $\geq$5. Developmental delay, intellectual disability and learning disability were found to be significantly associated with intractable epilepsy. A non significant trend was seen in those without febrile seizures towards seizure score <5. Those who had a later onset of unprovoked seizures were showing a trend towards seizure score $\geq$ 5. Any status epilepticus and multiple seizure types were favouring seizure score $\geq$5.

On comparing the seizure outcome, there was no significant difference between the various clinical phenotypes, presence of baseline developmental delay, regression, autism spectrum disorder, hyperactivity, ADHD, clustering of seizures, vaccine provoked seizures, type of seizure, sleep activation of seizures, MRI characteristics or need for polytherapy. Keto diet or delay in genetic testing had no contribution to seizure control in our study.

All 7 patients who were on Zonisamide were having seizure score > 5 which was found to be significant indicating that Zonisamide despite its multiple mechanisms of actions, including blockage of sodium channels, blockage of T type calcium channels and weak inhibition of carbonic anhydrase activity, may not improve seizure outcomes in DS. 2 patients who received sodium channel blockers were found to have intractable epilepsy. Medications found to be relatively beneficial and well tolerated included valproate, clobazam, levetiracetam and topiramate. Drugs which caused worsening were phenytoin (2 patients), carbamazepine (2

patients), oxcarbazepine (2 patients), perampanel (1 patient), topiramate (1 patient) and zonisamide (1 patient). Among the patients who were seizure free, combinations which contributed were valproate, clobazam, levetiracetam and topiramate and 30% of these patients were diagnosed to have DS and 70% to have DB phenotypes.

Seizure freedom vs ASM resistant epilepsy

Among those with and without seizure freedom, absence of febrile seizures was found to be significantly associated with seizure freedom. Those who had seizure freedom demonstrated age appropriate development. Language delay and learning disability were significantly higher in the ASM resistant epilepsy group, and there was a non significant propensity for intellectual disability and dependency in those with intractable epilepsy. Multivariate analysis was attempted but coherent models could not be generated possibly due to low sample size. There was a non significant trend for early age of onset of febrile seizures and multiple seizure types towards ASM resistant epilepsy . *SCN1A* positivity as well as truncating mutations also showed a trend towards no seizure freedom. Prior studies have shown that earlier onset of focal seizures with impaired awareness, status epilepticus, presence of motor disorder and interictal EEG abnormalities in the first year of life could predict a non favourable outcome whereas mutation type could not predict the outcome. (58)

Developmental outcomes

On comparing those who had developmental delay, developmental delay was significant in those who had febrile status when compared to those without febrile status and there was a non significant propensity for any episode of status epilepticus towards developmental delay. There was a trend towards developmental delay in patients with febrile seizures and in those with early age of onset of febrile seizures with clustering of febrile seizures. control of febrile seizures and especially proper management of status epilepticus could make a difference in the developmental outcome. Similar finding was seen in study by Brunklaus et al where status epilepticus was associated with worse developmental outcome.(58)

There was significant association between seizure freedom and development. Sixty percent of children who had seizure freedom had age appropriate development.

There were no significant differences in the EEG or MRI findings of patients with or without developmental delay. On multivariate analysis, age at onset of febrile seizures, clustering of febrile seizures, status epilepticus, number of antiseizure medications, seizure freedom were

not found to be significantly associated with developmental delay. Those who had developmental delay, they had a significant association with polytherapy. The mean number of antiseizure medications used in those with normal development was 2.8 vs 3.4 in those with delayed development, which is found to be significant as expected in view of higher number of ASM use in refractory seizures and the association between refractory seizures and developmental delay. Polypharmacy was found to cause significant cognitive impairment in prior studies (59) and being a modifiable factor, it is important to balance between the benefits and side effects. The impact of polypharmacy needs to emphasized in this group of patients and this is reflective of the need for developing newer approaches including drug repurposing and gene therapy approaches such as anti-sense oligonucleotide therapies (ASO) or genome editing using CRISPR technology as has been tried in other ion channelopathies. (60)

Delay in genetic diagnosis did not contribute to developmental outcome. There were no significant differences between developmental outcome among the various clinical phenotypes.

The clinical manifestations can vary widely across various missense mutations and it cannot be said that missense mutations cause mild phenotypes, as missense mutations in the pore forming region are found to cause severe phenotypes. Hence identification of key protein area may help in identifying the nature and significance of a particular mutation. (43) However we demonstrated a trend of truncating mutations producing more severe phenotypes. In view of the non-availability of approved therapeutic strategies like stiripenol, fenfluramine and cannabidiol in India, the most preferred combinations to treat these patients may include valproate, clobazam, levetiracetam and topiramate in variable combinations. Phenobarbitone can also be tried in case of refractory seizures. Sodium channel blockers has to be avoided as it can worsen the seizures. There is no definite evidence for Zonisamide from available literature.

Ketogenic diet has been found to be effective against all types of seizures in Dravet syndrome and has to be considered in all patients with refractory seizures. Our study was underpowered to conclude the utility of ketogenic diet, as there were only a few patients who could follow the diet.

In the current era of newer treatment options like gene modulatory therapies, it is important to determine the functional status of variants, ie, gain of function vs loss of function and it also helps to initiate early treatment so as to avoid further intellectual impairment.(61)

Antisense oligonucleotides (ASO) and Adeno-associated virus (AAV)-delivered gene modulation are the new therapeutic approaches for DS. These can cause irreversible exacerbation of gain of function variants and here comes the importance of assessing the functional status of new variants. (61) However, *SCN1A* coding sequence is long, exceeding the capacity for AAVs, restricting the rapid development of this technique. With the advent of CRISPR technology for genome editing, targeting DNA has become more efficient. (60) Table 4.2 summarizes our findings in light of other studies in published literature.

Table 7.2 Studies from various countries on Dravet genotype phenotype correlation

Country	Number of participants	Variables studied	Results
United Kingdom 2011	273 (single centre)	Truncating vs missense variants Genotype – phenotype corelation	- truncating mutations were associated with earlier mean onset of prolonged seizures, myoclonic seizures and atypical absence seizures - Missense mutations occurred most frequently in the voltage and ion-pore regions where changes in amino acid polarity were greater in the Dravet group compared to the genetic epilepsy with febrile seizures plus group (43)
United Kingdom 2012	241 (single centre)	Clinical, EEG, MRI characteristics of *SCN1A* mutation positive DS	-incidence is 1:40,900 - higher male to female ratio of 1.27:1 -clinical features predicting a worse developmental outcome included status epilepticus, interictal EEG abnormalities in the first year of life and motor disorder -no significant effect was seen for seizure precipitants, MRI abnormalities or mutation class (truncating versus missense) - Sodium valproate, benzodiazepines and topiramate were reported as being the most helpful medications (58)
China 2012	181 (single centre)	*SCN1A* gene mutations, inheritance pattern and genotype – phenotype corelation	Mutation rate of *SCN1A* in DS is about 70%. Majority are missense (47%) and truncation mutations (43%). Most are de novo. Phenotypes were mild or normal.(62)

France 2014	333 (multicentre)	Mutation spectrum associated with DS	-wide mutation spectrum of *SCN1A* associated with DS, including missense, nonsense, splice-site mutations, small deletions and insertions, and rare large-scale deletions - missense mutations were the most common mutation type identified -no difference in phenotypes among the various mutations (63)
Japan 2017	295 (multicentre)	Truncating vs missense variants	- incidence of DS in Japan - 1:45,800 -91.2% had denovo mutations - truncation variants are associated with a more rapid progression to severe phenotype (more severe intellectual disability) and the rate of progression is relatively consistent regardless of age of initial seizure onset - higher rate of febrile seizures in families with de novo mutations - stiripentol, topiramate, bromide, and levetiracetam appeared effective in patients with truncating variants and clonazepam, bromide, topiramate, and phenobarbital were effective in patients with missense variants (44)
China 2019	24 (single centre)	Genetic and phenotypic characteristics of *SCN1A*-related epilepsy	-descriptive study - age of epilepsy onset ranged from 2 months to 2 years and 9 months. Multiple seizure types were observed, 62.5% patients showed varying degrees of cognitive and motor development retardation, 91.7% patients had de novo mutations (64)

USA 2020	137 (single centre)	Genotype phenotype correlations in DS	- Retrospective study -No differences in the clinical profile or seizure outcome between missense or truncating variants
India 2022 (present study)	49 (single centre)	Missense vs truncating mutations, predictors of seizure freedom and developmental delay	-prospective cohort - children with truncating variants demonstrated a trend towards typical Dravet phenotypes, high likelihood of multiple seizure subtypes and higher proportion of ASM resistant epilepsy. -typical Dravet was found to have higher proportion of positive family history and a trend towards febrile status - Developmental delay, intellectual disability and learning disability were found to be significantly associated with intractable epilepsy -presence of febrile seizures, multifocal IEDs in EEG and truncating mutations were more seen in ASM resistant epilepsy -Developmental delay had significant association with febrile status and trend towards early onset febrile seizures and any episode of status epilepticus in DS/DB phenotypes (irrespective of febrile or afebrile status) - poor seizure score with zonisamide

Conclusion

- This is the first study from the subcontinent which has addressed the significance of variant subtypes among a cohort of children who met the clinical criteria for DS or DB phenotypes.
- Apart from *SCN1A* variants we also detected *SCN1B*, *GABRA1, GABRG2*, *PCDH19*, *CHD2*, among others associated with DS or DB phenotypes.
- There is no electroclinical or radiological parameter to differentiate between the phenotypes of missense vs truncating mutations.
- The severity of disease, seizure control, neurocognitive outcome do not vary between the two groups although children with missense variants demonstrated a trend towards Dravet borderline phenotypes, lower likelihood of multiple seizure subtypes and higher proportion were seizure free.
- Our study establishes that all though truncating variants demonstrated a non significant trend towards more severe phenotypes the impact of severe missense variants in south India needs to be reiterated on seizure and developmental outcomes.
- Our results challenge the notion that truncating variants alone have a more severe impact on long term seizure and developmental outcomes among children with this developmental epileptic encephalopathy and *SCN1A* mediated epilepsy is a prototype DEE irrespective of the variant subtype.
- It was found that family history of febrile seizures was found to be seen more with typical Dravet and a trend was seen among typical Dravet phenotype towards febrile status and truncating mutations whereas Dravet borderline phenotype had propensity towards clustering of seizures and missense *SCN1A* variants.
- A nonsignificant trend of febrile seizures, status epilepticus, multiple seizure types and later onset unprovoked seizures towards high seizure score was observed. Also generalised IEDs were more common among patients with seizure score $\geq$5.
- Developmental delay, intellectual disability and learning disability were found to be significantly associated with intractable epilepsy highlighting the importance of development of precision medicine-based approaches in this DEE.
- There was a nonsignificant trend for early age of onset of febrile seizures and multiple seizure types towards ASM resistant epilepsy, *SCN1A* positivity as well as truncating mutations also showed a trend towards ASM resistant epilepsy.
- Developmental delay had significant association with febrile status and a trend towards early onset febrile seizures and any episode of status epilepticus in DS/DB phenotypes (irrespective of febrile or afebrile status).

- Control of febrile seizures and especially proper management of status epilepticus could make a potential difference on developmental outcomes.
- We also demonstrated a significant association of poor seizure score with zonisamide which need further studies to confirm.

Strengths

- Ours is a prospectively maintained database with a sizeable number of patients
- This is the first study from Indian subcontinent on a sizeable cohort from a single centre.
- We could characterize the cohort into DS and DB phenotypes along with seizure and developmental outcomes which enabled bivariate comparisons.
- Our study emphaizses the relevance of robust clinical phenotyping and therapeutic decision making, with genetic test results being ancillary or supportive to seizure management decisions and prognostication.

Limitations

- Our study is limited by sample size, but this is an ongoing registry and around 12 patients could not be included in the study due to pending genetic reports and lack of 1 year follow up.
- Given the reported incidence of DS/DB phenotypes in population based studies of 1 20-40,000 (2) this is not surprising and our cohort represents the largest reported from a single centre in south India.
- Those numbers could have further impacted our results as the prevalence of *SCN1A* variants is marginally less than that of reported studies.(2)
- Another limitation was that with our centre being a referral centre, the numbers would not be a representative data of the general population.
- One of the challenges we had to face was the delay in genetic diagnosis, especially inability to uniformly undertake segregation analysis in the entire cohort to establish whether all variants are denovo or not.
- This is important given the prevalence of positive family history of any febrile seizure/epilepsy in our cohort with a sizeable proportion detected to have genetic variants.
- The interpretation of variants of unknown significance and non-availability of functional assays made us to rely on literature and insilico prediction models for

interpretation. We however followed the standardized methodological approach towards variant classification and interpretation.

- Another limitation is our reliance on clinical and Denver development quotients rather than structured estimations as done in neurodevelopmental clinics.
- Future studies need to inculcate these measures to enable quantification of domain specific developmental trajectories in this DEE.

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Appendix – 1

STRUCTURED PROFORMA

Demographic data:

Name

Age in months

Gender

Consanguinity

Number of siblings – how many affected with similar illness

Antenatal history – GDM, PIH, maternal infections, prior abortions, psychiatric illness, drug intake, reduced fetal movements

Timing of delivery – term/ preterm

Mode of delivery – vaginal/assisted vaginal/LSCS

Complications during delivery

Cry after birth – immediate/ delayed

Birth weight – normal / SGA

NICU stay /ventilatory support

Perinatal insults/antecedents – respiratory distress/pathological jaundice/neonatal meningitis/neonatal sepsis/hypoglycemia/hypocalcemia

Development history:

Development – normal /delayed

If delayed – domain affected – Global/gross motor/fine motor/language/social

Regression – if present – domain affected – Global/gross motor/fine motor/language/social

ASD - deficits in social communication, restricted and repetitive patterns of behaviour, highly restrictive and fixated interests

Activity – Normal / hyperactivity /hypoactivity

Family history – febrile seizures/ epilepsy / both

Seizure data:

Age of onset of febrile seizures

Type of FS – simple/complex

Classification of seizures – focal motor seizures : with and without impaired awareness, focal non motor seizures : with and without impaired awareness, focal to bilateral tonic clonic seizures, generalised motor seizures, generalised non motor seizures, unknown onset motor seizures, unknown onset non motor seizures, unknown onset unclassified seizures

If motor : tonic, clonic, tonic clonic, myoclonic, atonic, epileptic spasms, hyperkinetic, automatisms

If non motor : typical absences, atypical absences

Frequency of FS

Clustering of FS

Febrile status

Hospital stay – number of admissions, days of hospital stay

Medications used for febrile status

Route of administration of drug

Unprovoked seizures:

Age of onset of unprovoked seizures

Classification of seizures

Multiple seizure types

Clustering / status epilepticus – unprovoked

Hospitalisation data – number of admissions, days of stay

Any clustering / status epilepticus

Vaccine provoked seizures : vaccine, latency of vaccine provoked seizure

Periodicity of seizures

Sleep activation of seizures

Lateralisation and localisation based on history/ home video if available

Epilepsy diagnosis – focal/generalised/combined

Clinical impression of epileptic encephalopathy

Neuropsychiatric disturbances

Sleep disturbances

Respiratory/ Gastrointestinal disturbances

Academic performance

Examination:

General examination – head circumference: normal/microcephaly/macrocephaly

Facial dysmorphism / Neurocutaneous markers / Skeletal deformities

Speech

Visual regard / Auditory regard

Fundus / Squint/ Ophthalmoplegia

Motor: Tone/weakness/DTR/plantar

Ataxia / Gait / Movement disorders

Investigations: (available)

Lactate /pyruvate/ABG /uric acid /ammonia

Plasma amino acids/ urine organic acids

Csf study – if done, findings

EEG data:

Background activity

Interictal discharges – side, location

Multifocal IEDs

Generalised IEDs

Sleep – sleep architecture

PDR

Photosensitivity

GPFA

BA pattern

Ictus - onset, pattern, type of seizures recorded

MRI abnormalities:

Atrophy/ malformations/ FCD/ WM HI/ PVL/ Corpus callosal thinning

Developmental assessment:

Language delay – expressive / receptive (REELS)

VSMS - SQ

Intellectual disability

Learning disability

ADHD

ASD

DQ – developmental age / chronological age * 100 (gross motor / fine motor / social / language)

DQ – mild/moderate/severe

Dependency for ADL

Final diagnosis – Dravet/ Dravet borderline (DB-M, DB-SW, DB-O, DB-GTCS)

Treatment details:

Current ASM

ASM tried previously

Best combination

Drugs with worsening

Allergy

Side effects

Other medications: antipsychotics, pulse steroids, IVIG, vitamin supplements

Keto diet

Seizure score at baseline and at follow up : 0- Seizure free, off ASM, 1- Seizure free, need of ASM uncertain, 2- Seizure free, need ASM, 3-Auras only, 4- Non-disabling nocturnal Seizures only, 5:1-3 seizures/year, 6: 4-11 seizures/year, 7:1-3 seizures/month, 8: 1-6 seizures/week, 9:1-3 seizures/day, 10: 4-10 seizures /day, 11:>10 seizures/day, 12:Status, no barbiturate coma

Seizure freedom

Genetic data:

Test administered

Variant identified – ACMG criteria: Class 1- benign, Class 2 likely benign , Class 3 variant of uncertain significance (VUS), Class 4 likely pathogenic, Class 5 pathogenic

SCN1A variant – missense vs truncating

Location of variant – amino acid change

Functional status of variant – loss of function / gain of function

Novel variant

Other variants identified

Zygosity

Inheritance

Delay in genetic diagnosis

Stage of disease at enrolment and follow up – preseismic/ seismic/ post seismic

Current status of epilepsy - 1- epilepsy in remission with normal cognition

2- epilepsy in remission with developmental delay

3- intractable epilepsy with normal cognition

4- intractable epilepsy with developmental delay

Duration of followup

Appendix 2

INFORMED CONSENT

By signing this form, I agree that:

1) You have explained this study to me. You have answered all my questions.

2) You have explained the possible benefits and harms (if any) of this study.

3) I understand that I have the right to refuse to take part/ let my child take part in the study. I also have the right to withdraw/ take my child out of the study at any time.

4) My decision about my child taking part in the study will not affect my child's health care at the institute.

5) I am free now, and in the future, to ask questions about the study.

6) I have been told that my/ my child's medical records will be kept private. You will give no one information about my child, unless the law requires you to.

7) I have read and understood this consent form.

I give my consent for taking part myself/ letting my child _____take part in this study.

_____ _____

Printed name of parent/legal guardian Signature & date

_____ _____

Printed name of person who explained consent form Signature & date

_____ _____

Printed name of witness (if the patient/ parent/legal Guardian does not read English/Malayalam) Witness' signature & date

Apendix 3

ABBREVIATIONS

AAV- Adeno-associated virus

ABG – Arterial blood gas

ACMG - American College of Medical Genetics and Genomics

AD – Autosomal dominant

ADHD - Attention deficit hyperactivity disorder

ADL - Activities of daily living

ALDH7A1 - Aldehyde Dehydrogenase 7 Family Member A1

AR – Autosomal Recessive

ASD – Autism spectrum disorder

ASL- Arterial spin labeling

ASO - Antisense oligonucleotides (ASO)

BA – Burst Attenuation

BGA – Background activity

CA – Chronological age

CACNA1H - Calcium Voltage-Gated Channel Subunit Alpha1 H

CC – Corpus Callosum

CES – Clinical exome sequencing

CHD2 - Chromodomain Helicase DNA Binding Protein 2

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

CSF – Cerebrospinal fluid

DA – Developmental age

DB- Dravet borderline

DDST – Denver Developmental Screening Test

DEE - Developmental and epileptic encephalopathy

DPT – Diphtheria, Pertussis, Tetanus

DQ – Developmental quotient

DS - Dravet syndrome

DSM - Diagnostic and Statistical Manual of Mental Disorders

DTR – Deep tendon reflexes

EEG – Electroencephalogram

FCD – Focal Cortical Dysplasia

FS – Febrile seizure

GABA - Gamma-Aminobutyric Acid

GABRA1 - Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1

GABRG2- Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma2

GABRD- Gamma-Aminobutyric Acid Type A Receptor Subunit Delta

GDM – Gestational diabetes mellitus

GEFS+ - Generalized epilepsy with febrile seizures plus

GPFA – Generalised paroxysmal fast activity

GPR98 - G protein-coupled receptor 98

ICEGTC - Intractable childhood epilepsies with frequent generalized tonic clonic seizures

ID – Intellectual disability

IEDs - Interictal Epileptiform Dischargers

ILAE - International League Against Epilepsy

IPV – Injectable polio vaccine

IVIG – Intravenous Immunoglobulin

KD – Ketogenic diet

LSCS - Lower segment Caesarean section

LGS - Lennox-Gastaut Syndrome

MLPA - Multiplex-ligation dependent probe amplification

MRI – Magnetic Resonance Imaging

NGS – Next generation sequencing

NICU – Neonatal intensive care unit

OT – Occupational therapy

P/LP - Pathogenic/ likely pathogenic variants

PCDH19 - Protocadherin 19

PCR – Polymerase Chain Reaction

PDR – Posterior dominant rhythm

PHR – Posterior head region

PIH – Pregnancy induced hypertension

PVL – Periventricular leukomalacia

REELS - Receptive Expressive Emergent Language test

SCN- Sodium channel

SMEB- Borderline severe myoclonic epilepsy of infancy

SMEI - Severe myoclonic epilepsy of infancy

SQ – Social Quotient

STXBP1 - Syntaxin-binding protein 1

VEEG – Video electroencephalogram

VSMS - Vineland Social Maturity Scale

VUS - Variant of uncertain significance

WES – Whole exome sequencing

WISC- Wechsler Intelligence Scale for Children

WM HI – White matter hyperintensity

WT – wild type

Appendix 4

LIST OF TABLES

Appendix 4

LIST OF FIGURES

Ethics Committee Approval letter

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेंद्रम - 695 011, केरल, भारत
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
TRIVANDRUM - 695 011, KERALA, INDIA
(एक राष्ट्रीय महत्व का संस्थान, विज्ञान एवं प्रौद्योगिकी विभाग, भारत सरकार)
(An Institution of National Importance, Department of Science and Technology, Government of India)
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DST@ 1971-2021 DEPARTMENT OF SCIENCE & TECHNOLOGY

Institutional Ethics Committee

(IEC Regn No. ECR/189/Inst/KL/2013/RR-16)

SCT/IEC/1595 /DECEMBER-2020 16.12.2020

Dr. Krishna S
Senior Resident
Department of Neurology
SCTIMST, Thiruvananthapuram

Dear Dr.Krishna,

Thank you for submitting documents related to your proposal titled "**GENOTYPE-PHENOTYPE CORRELATIONS AND PREDICTORS OF COGNITIVE OUTCOMES IN DRAVET SYNDROME & DRAVET BORDERLINE PHENOTYPES** (IEC/1595)" to the IEC for review.

The following documents were reviewed:

1. Check list
2. Proposal
3. IEC Application Form
4. Covering letter addressed to the Chairperson, IEC, SCTIMST dated 27.08.2020 by the HOD
5. Covering letter addressed to Chairperson, IEC, SCTIMST dated 27.08.2020 by Co.PI Dr.Ramshekhar Menon
6. Proforma
7. TAC Approval with Comments and responses
8. Dean's signature form
9. Subject information and informed consent title
10. Informed Consent (English)
11. Informed Consent (Malayalam)
12. CV of Dr.Krishna with TCMC number
13. CV of Dr.Ramshekhar Menon with TCMC number
14. CV of Dr.Ashalatha A, with TCMC number
15. CV of Dr.Soumya Sundaran with TCMC number
16. Covering letter from PI addressed to Chairperson, IEC, SCTIMST dated 05/09/2020

The following members of the Students Sub-Committee of the Institutional Ethics Committee participated in the discussions held between August 23-October 29, 2020 at the offices and residences of the members

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. R V G Menon	M Tech, PhD	Male	Lay Person (Chairman)	No
2.	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
3.	Dr. Kala Kesavan. P	MBBS, MD	Female	Basic Medical Scientist	No
4.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
5.	Dr. Rema M. N	MD	Female	Basic Medical Scientist	No
6.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
7.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision
The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

Plagiarism Check Report

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