Genotype-phenotype correlates of infantile-onset developmental & epileptic encephalopathy syndromes in South India: a single centre experience

ABSTRACT :

Introduction:

A paucity of literature exists on genotype- phenotype correlates of 'unknown-etiology' infantile-onset developmental-epileptic encephalopathies (DEE) from India. The primary objective was to explore the yield of genetic testing in identifying potential disease causing variants in electro-clinical phenotypes of DEE

Methods:

An observational hospital-based study was undertaken on children with unexplained refractory seizure-onset ≤12 months age and developmental delay, whose families consented and underwent genetic testing during a three year time period (2016-2018) by next-generation sequencing (NGS) or multiplex ligand protein amplification. Yield was considered based on demonstration of pathogenic/likely pathogenic variants only and variants of unknown significance (VUS) were documented.

Results:

Pathogenic/likely pathogenic variants were identified in 26 (31.7%) out of 82 children with DEE. These included those variants responsible for primarily DEE- 21(76.7%); neuro-metabolic disorders- 3(18.6%) and chromosomal deletions- 2(4.7%). Of these patients, early-infantile epilepsy onset ≤ 6months age was noted in 22(84.6%). The DEE studied included Ohtahara syndrome associated with *STXBP1* and *SCN8A* variants with yield of 50% (2/4 tested); early myoclonic encephalopathy (no yield in 2); West syndrome with *CDKL5*, yield of 13.3% (2/15 tested); epilepsy of infancy with migrating partial seizures due to *CACNA1A* and *KCNT1* variants, yield of 67% (2/3 tested); DEE-unclassified with *KCNQ2, AP3B2, ZEB2,* metabolic variants (*SUOX, ALDH7A1, GLDC*) and chromosome deletions (chr 1p36, chr2q24.3); yield of 32% (8/25 tested). Patients with Dravet syndrome/Dravet-like phenotypes (N=33) had variants in *SCN1A* (N=10), *SCN1B, CHD2*; yield of 36.4% (12/33 tested; 57.1% from NGS). Eighteen patients with potential variants (*SCN1A, SCN2A, SCN8A, KCNQ2, ALDH7A1* which also included VUS) could be offered targeted therapy.

Conclusions:

Our study confirms a good yield of genetic testing in neonatal and infantile-onset DEE provided robust phenotyping of infants is attempted with prognostic and therapeutic implications, particularly relevant to centres with resource constraints.

1. Introduction

Infantile epileptic encephalopathies include various phenotypes of epileptic encephalopathies (EE), which are characterized by seizure-onset before 12 months of age.(Zhang et al., 2017) The majority of EE have onset ≤ 6 months of age and are referred to as early-onset EE (EOEE) or early infantile EE (EIEE).(Hwang & Kwon, 2015; Nieh & Sherr, 2014)The syndromes include phenotypes with refractory seizures often accompanied by frequent focal, multifocal or generalized epileptiform abnormalities with age-inappropriate ontogeny on electroencephalography (EEG), and developmental delay (DD), regression or intellectual disability (ID).(Berg et al., 2010; Nieh & Sherr, 2014) EIEE include Ohtahara syndrome (OS), West syndrome (WS), early myoclonic encephalopathy (EME), epilepsy of infancy with migrating focal seizures (EIMFS), and Dravet syndrome (DS) as well as refractory focal or generalized epilepsy with developmental impairment which cannot be classified into named syndromes but may broadly come under the description of developmental and epileptic encephalopathies (DEE).(Kalser & Cross, 2018; Scheffer et al., 2017) Identifiable primary causes include structural, neurodegenerative, metabolic, pathogenic copy number variants, or chromosomal deletion/duplication syndromes. Increasingly, a number of novel and *denovo* genetic causes are being identified in childhood epilepsy, especially DEE of uncertain aetiology.(Alam & Lux, 2012; McTague, Howell, Cross, Kurian, & Scheffer, 2016) DEE is a genetically heterogeneous disorder: over 100 genes have been suggested to be involved in the aetiology of these syndromes.(McTague et al., 2016) Many DEE cases are sporadic occurring in patients with no family history of seizures or epilepsy.(Epi et al., 2013) Next-generation sequencing (NGS) and whole-exome sequencing (WES) allow the analysis of a variety of genes simultaneously, which is very useful in large sample analysis or multi-gene analysis. In one study, 265 monogenic epilepsy-associated genes were sequenced using targeted NGS in 33 patients with various well-phenotyped epilepsy syndromes and 48% were shown to have disease-causing variants.(Lemke et al., 2012) Employing the more expensive whole genome sequencing identified variants in four of the six patients with DEE. (Martin et al., 2014)Another study using NGS suggested a diagnostic yield of 28% for an underlying genetic cause in patients with EE in a retrospective cohort study with aetiologies including

metabolic disorders, pathogenic copy number variants (CNVs) and variants in *SCN1A, SCN2A, SCN8A, KCNQ2, STXBP1, PCDH19,* and *SLC9A6*genes.(Mercimek-Mahmutoglu et al., 2015)This information is lacking from the subcontinent wherein the advent of disease-specific panels has largely transformed diagnostics although challenges remain with regard to commercial costs, variant curation, absence of local population-based data, clinical interpretation and reluctance of unaffected family members to undergo testing.(Ganapathy et al., 2019)

With this background, we undertook this study with the following objectives:

1) To study the yield of genetic testing using commercially available epilepsy gene panels and/or MLPA in the broad category of DEE in a south Indian cohort, in terms of prevalence of potentially pathogenic variants with added documentation of variants of unknown significance (VUS) which may have clinical implications.

2) To qualitatively assess variant subtypes with estimates of pathogenicity in various phenotypes of DEE

2. Materials and methods

2.1. Study design and subjects

This hospital based retrospective observational study was conducted at a comprehensive epilepsy care referral centre in Trivandrum, Kerala, India. Patients with DEE or drug-resistant epilepsy of uncertain or unknown etiology with unprovoked seizure onset at or under 12 months of age with/without co-existent developmental delay or regression and whose families consented and underwent genetic testing between Jan 2016 to Dec 2018 were included. Electro-clinical syndromes and DEE were defined and classified as per ILAE criteria.(Berg et al., 2010; Scheffer et al., 2017) Detailed inclusion criteria for each electro-clinical syndrome with representative electroencephalographic (EEG) signatures are depicted in **Appendix 1**. We excluded children with Down Syndrome or Trisomies, first unprovoked or fever-provoked seizure onset beyond 12 months age, surgically-remediable syndromes, neuro-cutaneous syndromes, progressive myoclonus epilepsy syndromes or progressive symptomatic epilepsies secondary to neurodegenerative, leukopoliodystrophies or storage disorders. Metabolic disorders were screened for using plasma tandem mass spectroscopy for amino acids, organic acid and carnitine metabolites, serum homocysteine, serum copper and ceruloplasmin levels, CSF glucose, CSF lactate: serum pyruvate ratios and, in patients with co-existent movement disorders, plasma and urine neopterin and biopterine levels.

The demographic details and detailed clinical history of seizures including their response to AEDs were documented. Developmental age, video electroencephalography (EEG) and magnetic resonance imaging (MRI) results were evaluated. Developmental assessments were done in terms of best developmental age on revised Denver Development Scale. (*Denver II Technical Manual.* 1990;Denver Developmental Materials, Inc.) The Institutional Ethics Committee approved this retrospective study.

2.2. Genetic testing

Subjects evaluated by NGS or Multiplex Ligation-dependent Probe Amplification (MLPA) were included in the analysis. The techniques implemented for both tools are in Appendix A. The methods for library preparation, multi-gene panel sequencing, data analysis and variant interpretation has been detailed previously.(Ganapathy et al., 2019) Genetic test results were reported based on the recommendations of American College of Medical Genetics according to which, based on level of evidence a score is assigned. (Richards et al., 2015) A variant is defined as a change in a gene which could be pathogenic (P- disease causing variation), likely pathogenic (LP- very likely to contribute to disease, but insufficient scientific evidence for conclusive proof) , VUS (difficult to classify as pathogenic or benign based on current available scientific evidence) or variant of uncertain significance with probable damaging effect (VUS-D; identified variant alters a conserved residue and is predicted to be damaging to the protein function from atleast 3 *insilico* studies, without sufficient clinical evidence).Variants detected on NGS were confirmed by Sanger sequencing although segregation analysis could be completed in only 4 probands. Electro-clinical and genetic correlations were derived for both known and novel variants which were elicited from the 1000 genome, ExAC/GNOMAD, ClinVar, OMIM, GWAS and HGMDdatabases. The genome build hg19/GRCh37 was used for determining the chromosomal coordinates of variants. A variant was considered to be novel if it was previously unreported in these databases. Yield of testing was ascertained based on identification of *pathogenic* or *likely pathogenic* variants only.

3. Results

3.1. Patient profiles

A total of 82 patients with DEE underwent genetic testing during the three year time period (2016-2018) and potential variants were noted in 43(52.4%) patients. Early infantile onset of epilepsy was noted in 36/43 [neonatal 14 (32.6%), 1-6 months in 22 (51.2%)] and onset > 6months in 7 (16.2%). The yield of pathogenic/likely pathogenic variants were identified in 26 (31.7%), VUS-D in 8 (9.8%)

and VUS in 8(9.8%) and a benign susceptibility variant in 1(1.2%) while testing did not identify any variants in 39 (47.6%). Thirteen variants (30.2%) were identified as novel. Pathogenic/likely pathogenic variants thus constituted 60.5% of the variants identified overall. Early-infantile epilepsy onset was noted in 22 out of these 26 patients (84.6%). Genetic testing was done by NGS in 69 patients and using MLPA probes in 13 patients (12 for *SCN1A* deletion/duplication analysis and 1 for confirmation of FISH findings). The patients with variants with potential phenotype correlations as evident on online databases such as OMIM/ClinVar/GNOMAD were divided into the following groups: A)Genetic DEE including DS- 33 (76.7%); B) neuro-metabolic variats-8 (18.6%) & C) chromosomal deletions-2(4.7%). The most common variant types included 29 missense substitutions (67.4%) and 16 truncating, including compound heterozygous variants [37.2%; non-sense resulting in a premature stop codon in 4 (9.3%), single exon deletions 6 (14%), multiple exon deletions in 2 (4.7%),insertions/duplications in 3 (7%) and splice-site variants in 1 (2.3%)]. The distribution with regard to pathogenic/likely pathogenic variants is highlighted in **Figure 1 and Tables 1-4** while patients with VUS are detailed in the **Supplementary Table.**

3.2. Genetic DEE (non-DS phenotypes):

There were 17 patients (37.5%) in this group. Their clinical, biochemical, neuroimaging, and molecular genetic investigations are summarized in Table 1. Inheritance patterns were autosomal dominant (AD) in 46%, X-linked (XLR) in 26.6%, autosomal recessive (AR) in 6.6%, and mitochondrial (MT) in 6.6% of the patients. Denovo inheritance was confirmed in 3 patients. DEE in this group included OS**(Table 1A)**(N=2/4 tested positive for likely pathogenic/pathogenic variants out of 4 patients; yield=50%); EME (none out of 2; homoplasmic mitochondrial DNA VUS identified), WS (N=2/15; 13.3%)**(Table 1C)** and EIMFS (2/3;67%)**(Table 1B)**.The yield of pathogenic or likely pathogenic variants in other unclassified DEE **(Tables 1C, 2, 3)**was 8/25 (32%), which included variants associated with metabolic disorders (3) and chromosomal deletions (2).

Seizure onset was in the neonatal period in 10 out of 17 (52.9%) of the patients (*STXBP1, SCN2A, SCN8A, MT-ATP6, CACNA1A, KCNQ2* and *AP3B2* variants) of which 5 were pathogenic variants. Heterozygous missense variants were noted in 12/17 patients (70.5%) and truncation variants in 5 (29.4%) patients; denovo variants were established by segregation in 3(17.6%). These were deemed pathogenic/likely pathogenic in 9 (52.9%) and VUS with possible damaging effect in 3 (17.7%) and VUS with phenotypes similar to literature reports in 5 (29.4%). High dose sodium channel blockers (SCB) benefited the infants with *SCN2A* and *SCN8A* encephalopathy whereas quinidine could not be procured for the infant with *KCNT1* encephalopathy due to non-availability in the country.

Table 1A. Clinical, EEG and genetic profiles of patients with pathogenic/likely pathogenic variants causing DEE and suppression-burst patterns

Table 1B: Pathogenic/likely pathogenic variants causing epilepsy of infancy with migrating focal seizures (EIMFS)

Table 1C: Pathogenic/likely pathogenic variants causing other distinctive DEE including WS& DEE-unclassified

Supplementary Table: DEE phenotypes with VUS/VUS-D identified

3.3. Potential neuro-metabolic variants in DEE

The inheritance pattern was autosomal recessive in all patients, with one infant having sulphite oxidase deficiency due to compound heterozygous variations in *SUOX* gene. Seizure onset was in the first week after birth in 50% of the patients (sulfite oxidase deficiency, pyridoxine dependent epilepsy, Menke's disease and dihydrolipoamide dehydrogenase deficiency which leads to defective function of three mitochondrial enzyme complexes). Epilepsy was in remission in 2 patients (arginosuccinatelyase deficiency, D2-hydroxyglutaricaciduria) in addition to the 2 children with pyridoxine dependent epilepsy who had significant improvement with developmental normalisation on pyridoxine(10mg/kg/day) supplementation. The patients with glycine encephalopathy and D2-hydroxy glutaric aciduria benefited in terms of seizure control with dietary modification and co-factor supplementation.

Table 2 & supplementary table: Clinico-electrophysiological and genetic profile of patients with neuro-metabolic variants

3.4. Chromosomal abnormalities

Two patients with DEE-unclassified with accompanying dysmorphias (5%) were found to have major chromosome deletions, namely 2q24.3 microdeletion syndrome involving major *SCN* gene clusters (detected on NGS) and 1p36 deletion syndrome (detected on FISH and confirmed on MLPA).

Table 3. Clinico-electrophysiological and genetic profile of patients with chromosomal abnormalities

3.5.Dravet syndrome and Dravet-like phenotypes

There were 16 patients (total N=33; 48.5%) in this group with variants. Twelve patients who were subjected to only MLPA in view of targeted *SCN1A* analysis had no yield from this test. Twelve out of 21 patients who underwent NGS were deemed to have pathogenic/likely pathogenic variants (yield of 57.1%) and VUS-D in 3 (14.3%) and a benign susceptibility to epilepsy variant in 1 (4.8%). Variants included heterozygous missense variants in 10 patients (9 in *SCN1A, 1 CACNA1H*), homozygous missense variant in *SCN1B* in 1 patient, truncation variants in 5 patients (including CHD2 and intronic splice-site variant in *SCN1A*).Two variants in *CHD2* and *CACNA1H* were identified having Dravet-like phenotypes with concurrent photosensitivity, with the latter on segregation concluded to be a benign variant, although described in families with susceptibility for genetic generalized epilepsy.(**Table 4 & supplementary table for VUS**) Epilepsy was in remission in one patient with, frequent seizures persisted in 11 and seizures were infrequent in four patients. Avoidance of exclusive SCB like lamotrigine and carbamazepine could be planned for *SCN1A* patients. Stiripentol was recommended to families with refractory *SCN1A*-related phenotypes but only 1 child received this medication (not licensed for use in India) with significant benefit in seizure control.**Table 4A Dravet syndrome – pathogenic/likely pathogenic missense variants:**

Table 4B- Dravet syndrome – pathogenic/likely pathogenic truncation variants :

DISCUSSION:

Our study identified pathogenic/likely pathogenic variants in nearly one-third of patients diagnosed to have DEE over a 3 year period, with a majority presenting in early infancy. The yield exceeded 30% in most DEE syndromes (including unclassified subtypes) with the notable exception of WS. Besides diagnostic, prognostic and predictive utility in the entire cohort, this had therapeutic implications in ion-channelopathies and neuro-metabolic disorders all of which constituted around 50% of the cohort. Challenges encountered at our centre with the only recent availability of commercial epilepsy gene panels include a time lag to diagnosis (mean= 2.5 years; ranging from 1 month to 6.5 years), inability to uniformly undertake trio testing through the entire cohort to establish if variants are *denovo*, interpretation of variants of unknown significance and non-availability of functional assays due to which reliance on literature and *insilico* prediction models were paramount to our interpretation. We however followed a standardized methodological approach towards variant classification.

Following recognition for a genetic cause for EE in 2001 in DS, with the finding that all 7 children in a series had a *de novo SCN1A* variant, genetic testing in epilepsy and EE has advanced by leaps and bounds.(Claes et al., 2001) The advent of chromosomal microarray and NGS of multiple genes has led to a rapid growth in identification of potentially pathogenic variants for EE.(Epi et al., 2013; Veeramah et al., 2013) In the case of DS, as noted by us, pathogenic/likely pathogenic variants were identified in nearly 60% of the group using NGS with more than two-thirds having missense variants and remaining having truncation variants as has been noted previously.(Zuberi et al., 2011) This was after exclusion of patients evaluated by MLPA which is useful to identify only large deletions/duplications as seen in the patient with chromosomal deletions, which had no yield in DS. Our lower yield in comparison to the 70-80% yield of *SCN1A* variants reported in DS literature is potentially due to inclusion of patients with Dravet-like phenotypes and also because we could not retest patients who were MLPA negative with NGS or *SCN1A* sequencing despite a high index of clinical suspicion.(Brunklaus et al., 2013; Hildebrand et al., 2013) The phenotypic picture overall was crucial for interpretation of the importance of missense variants in the absence of functional assays in our study, and dictated our therapeutic approach. Other 'channelopathy' variants identified by us which have potential therapeutic implications in EE based on available published data included *SCN1B, SCN2A, SCN8A, KCNT1, KCNQ2* and *CACNA1A*.(McTague et al., 2016)

Genetic heterogeneity was noted in every electro-clinical phenotype in our series. Even among DS patients who had a yield, in which more than 80% of patients had *SCN1A* variants, other genes (eg. *SCN1B* and *CHD2* both of which were associated with prominent photosensitivity with eyelid myoclonia) accounted for a small proportion of cases.(Carvill et al., 2014) Although *SCN1A* gene testing alone may prove cost-effective in typical DS, detection of non-*SCN1A* genes is an added advantage of NGS. Similar heterogeneity was noted by us in OS with suppression-burst (*SCN2A, SCN8A, STXBP1*), EIMFS (*CACNA1A & KCNT1*) however it was maximal for WS (*CDKL5, ARHGEF9* and *GRIN2A* variants) and DEE-unclassified. Often a few cases of a novel genetic encephalopathy are initially recognised, and further studies are needed to confirm the role of the newly identified gene as causative as was shown recently with *CHD2*-mediated epilepsy although phenotypic matches on OMIM are pertinent.(Carvill et al., 2013) Many genes have been identified for classic DEE such as WS (Epi et al., 2013; Michaud et al., 2014) and OS with most genes associated with only a limited number of cases. (McTague et al., 2016). DEE-unclassified predominated in numbers in our series, characterized by infantile-onset of multiple seizure types, frequent multifocal or generalized epileptiform activity without the typical signatures of defined electro-clinical syndromes and with poor developmental progress. Within this heterogeneous group, specific genetic DEE have emerged through careful phenotyping of cohorts with variants of the same gene; these findings will enable recognition of the phenotype in the future as has

been shown with *SYNGAP1, CHD2* as identified in our series and *SLC6A1*encephalopathies.(Carvill et al., 2015; Thomas et al., 2015; Vlaskamp et al., 2019) Unique clinical, EEG, or MRI features of each phenotype caused by the various variants responsible for DEE-unclassified are likely to emerge in future with pragmatic testing, standardization of variant classification (using data on frequency in general population, inheritance, type of variants and prediction of functional affect on proteins with phenotyping) and reporting of similar cases across centers. . Although variants in several genes might result in identical DEE, dysfunction of various genes has been suggested to lead to disruption of physiological pathways in ontogeny and synaptogenesis that result in a given phenotype.(Paciorkowski et al., 2011)

The yield of a gene panel is often determined by the number of genes included in the panel and over time the commercially available panels used in our study varied from 80-199, gradually increasing over time with addition from literature. Our yield of 32% is comparable to the results of a recent prospective multi-centric study which demonstrated that 24% children with epilepsy had a diagnostic genetic finding, with monogenic causes identified in 1 per 2120 live births.(Symonds et al., 2019) The variant profile identified by us was similar to this study with the exception of absence of *PCDH19* and *SLC2A1* variants in our series. *SLC2A1* variants identified by us were not reported here given our inclusion criteria and in our experience, children with GLUT-1 deficiency had epilepsy onset >1 year of age as opposed to what was seen in the study. Regional demographic and phenotypic variations are thus expected although the overall distribution and genotype spectrum in our series is similar to the Western world. Our yield in DEE is lower than that of whole genome studies wherein the utility has been shown to approach 67%.(Martin et al., 2014). Potential mechanisms of pathogenicity in the absence of family history in dominant variants include denovo variants, genetic mosaicism, variable penetrance, phenotypic variability and in case of X-linked dominant disorders like *CDKL5*, skewed X-chromosome inactivation.(Biesecker & Spinner, 2013; Franco & Ballabio, 2006; Tarailo-Graovac, Zhu, Matthews, van Karnebeek, & Wasserman, 2017) The gold standard would be for all novel variants, even in known genes, to undergo functional assessment in a model system, which in our case was based on understanding of in-silico prediction in the results after confirmation of the variants on Sanger sequencing. Given the prevalence of heterozygous missense variants in our series it was particularly relevant for us to establish whether the variants were *de-novo* or inherited which was not possible given financial and logistic limitations. It is well known that AD-inherited variants may be causative in the absence of positive family history in view of variable penetrance.(Helbig, Heinzen, Mefford, & International League Against Epilepsy Genetics, 2018). Whereas variants can be fixed in certain populations due to the phenomenon of "genetic drift",(Kosmicki et al., 2017) one also needs to understand that many variants do not have sufficient evidence in the literature to allow classification and this aspect is discussed with families at the time of counselling. *Denovo* inheritance (which could be established in only 4 probands after testing of parents in our series) could not be uniformly assessed in our study in the absence of segregation analysis in the entire cohort. Not surprisingly, the presence of numerous disease-associated de novo variants in variant databases of the general population has generated doubt about their pathogenicity.(Kosmicki et al., 2017) Consequently, the true causality of many previously identified disease-associated variants has been recently questioned.(Manrai et al., 2016)These findings underscore the need for use of large control cohorts from the same region when studying genetic basis of common diseases but may not necessarily apply to rare disorders such as DEE.

The 19% of patients identified in our series with variants responsible for neurometabolic disorders is higher than that shown in literature.(Mercimek-Mahmutoglu et al., 2015).More than 200 metabolic disorders can present with epilepsy and EE and the majority of these disorders have targeted treatments.(Rahman & Rahman, 2019; Wolf, Bast, & Surtees, 2005) Many biochemical screening tests have been used to diagnose specific IMD or pathway defects related to those disorders, but without suggestive history or phenotypic features, the diagnostic yield of these investigations is very low. In our retrospective cohort study, only2 of the disorders were identified or suspected in the patients before-hand (based on plasma glycine levels in a child with myoclonic epilepsy-ataxia spectrum and pyridoxine response in status epilepticus in pyridoxine dependent epilepsy) with the tests in the remaining subjects through biochemical methods could not be conducted as these are not readily available in the country or are prohibitively expensive. It remains debatable whether all subjects with DEE should be subjected to the comprehensive array of tests advised by most centres in terms of cost-effectiveness over and above targeted testing following the genetic test results.(van Karnebeek et al., 2018) The remaining non-biochemically confirmed patients had phenotypic features consistent with previous reports. While our approach may be fraught with risk of over-interpretation in the absence of biochemical confirmation, it has the potential to guide investigations in neurometabolic epilepsy. The chromosomal deletion syndromes identified in our series in chromosomes 2 and 1 represents a potential utility in identifying deletions in multiple epilepsy genes that cluster around one locus of a chromosome although these are equally likely to be apparent on micro-array or MLPA studies. While micro-array studies are of high yield in EE associated with prominent dysmorphias or ID, doing this in sequence before NGS/MLPA studies would have exponentially increased the cost of investigations at our centre which has to be borne by families and is not state-funded presently, especially as the tests are not available in-house.

An obvious limitation is, given the retrospective design, the absence of genetic data of all consecutive patients with DEE over 3 years as we included the data of only children whose parents consented for panel-based genetic testing during evaluation. In the absence of uniform trios-testing we were unable to establish what proportion of our cohort had causative *denovo* variants which is due to factors such as difficulty in convincing parents in our society where there is considerable stigma attached and potential expenses involved, which makes families hesitant. Specific metabolic testing was not available for some of the metabolic gene variants. Additionally, a distinct referral bias is apparent in our study in terms of the profile of patients evaluated at our comprehensive epilepsy care centre where referrals of patients with drug resistant epilepsy and EE are weighted towards pre-surgical evaluation for potential epilepsy surgery. It is possible that some of our children with surgically amenable syndromes like malformations of cortical development or MRI negative focal drug resistant epilepsy may harbour a somatic variant which can impact prognosis as has been shown in recent literature and this could contribute to an under-estimation of yield. (Garcia et al., 2020; Winawer et al., 2018) Incidence-prevalence estimates and true measures of yield of genetic testing require prospective multi-centric data as was detailed in the recent Scottish study.(Symonds et al., 2019)

Conclusions:

Our observational study which is the first of its kind from the Indian sub-continent reports a diagnostic yield of 31.7% for an underlying genetic cause in patients DEE. The results in terms of yield are broadly similar to a Western population. These variants included *SCN1A, SCN8A, KCNT1, CACNA1A, KCNQ2, CDKL5,AP3B2, ZEB2, SCN1B, CHD2,* genes responsible for inherited metabolic disorders and chromosomal deletions. Additionally, identification of certain VUS such as *SCN2A, ALDH7A1* had therapeutic implications. We recommend that targeted next-generation sequencing be ordered for the identification of underlying genetic causes of DEE in patients with normal MRI and results of metabolic tests, even in the absence of a well-defined electro-clinical phenotype. Variant interpretation should be based on the understanding that the genetic cause of the less well-defined syndromes is still uncertain. Future research will expand our understanding of the relevance of the unique variant to its contribution towards the network dysfunction that leads to DEE. With many gene-specific clinical trials on disease modifying therapies now in development (for which it will be critical to have a genetic diagnosis to enable enrollment), including anti-sense oligonucleotide therapy, larger multi-centric data assimilation is the way forward.

Legend to Figure 1: Frequency of pathogenic/likely pathogenic, variants of unknown significance (VUS), VUS with damaging effect (VUS-D) and benign variants among DEE phenotypes (N=43; EME-early myoclonic encephalopathy; EIMFS- epilepsy of infancy with migrating focal seizures; DEE- developmental & epileptic encephalopathies)

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