Delineation of the Genetic Causes of Complex Epilepsies in South African Paediatric Patients

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TABLE OF CONTENTS

LIST OF TABLES

TABLES IN PUBLICATIONS:

European Journal of Epilepsy - Seizure:

Genetics in Medicine:

TABLES IN SUPPLEMENTAL INFORMATION:

LIST OF FIGURES

FIGURES IN PUBLICATIONS:

***Frontiers in Neurology**:*

***Genetics in Medicine**:*

FIGURES IN SUPPLEMENTAL INFORMATION:

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ETHICAL APPROVAL

This research was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 232/2015) and was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent (and assent, where appropriate) was obtained from probands and parents, prior to participation in the study. Copies of the consent/assent forms and current HREC approval are included in the Appendix of this thesis.

DECLARATION ON THE INCLUSION OF PUBLICATIONS IN A PHD THESIS

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the following publication(s):

1. Esterhuizen AI, Carvill GL, Ramesar RS, Kariuki SM, Newton CR, Poduri A, et al. *Clinical application of epilepsy genetics in Africa: Is now the time?* Front Neurol [Internet]. 2018 May 2 [cited 2018 May 10];9(MAY):276. Available from: http://journal.frontiersin.org/article/10.3389/fneur.2018.00276/full.

2. Esterhuizen AI, Carvill GL, Fieggen K, McIntosh C, Ramesar RS, Wilmshurst JM. *Importance of genetic diagnosis in the management of early-onset epilepsies.* SAMJ South African Med J [Internet]. 2021;111:8–9. Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0256-95742021000100005&nrm=iso.

3. Esterhuizen AI, Mefford HC, Ramesar RS, Wang S, Carvill GL, Wilmshurst JM. *Dravet Syndrome in South African infants: Tools for an Early Diagnosis.* Seizure [Internet]. 2018 Nov 1 [cited 2022 Jun 14];62:99–105. Available from: https://pubmed.ncbi.nlm.nih.gov/30321769/.

4. Esterhuizen AI, Tiffin N, Riordan G, Wessels M, Burman RJ, Aziz MC, et al. *Precision medicine for developmental and epileptic encephalopathies in Africa—strategies for a resource-limited setting.* Genet Med [Internet]. 2022 Dec [cited 2022 Dec 12];0(0). Available from: http://www.gimjournal.org/article/S1098360022010012/fulltext.

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LIST OF ABBREVIATIONS

Adeno-Associated Virus vectors	AAV vectors
African Genome Variation Project	AGV
Age At seizure Onset	AAO
American College of Medical Genetics and Genomics and the Association of Molecular Pathology	ACMG/AMP
Angelman Syndrome	AS
Antiepileptic Drugs	AED
Antiseizure Medications	ASMs
Antisense Oligonucleotide	ASO
Attention Difficulties	AD
Autism Spectrum Disorders	ASD
Autosomal Dominant	AD
Autosomal Dominant Nocturnal Frontal Lobe Epilepsy	ADNFLE
Autosomal Recessive	AR
Benign Familial Neonatal/Infantile Epilepsy	BFN/IE
Central Nervous System	CNS
Chromosomal Microarray	CMA
Clustered Regularly Interspaced Short Palindromic Repeats	CRISPR
Compound Heterozygous	comp het
Copy Number Variant	CNV
Developmental and Epileptic Encephalopathies	DEE
Developmental Delay	DD
Dravet Syndrome	DS
Duchenne Muscular Dystrophy	DMD
Early infantile epileptic encephalopathy	EIEE
Early Onset Epileptic Encephalopathy	EOEE
Electroencephalography	EEG
Epilepsy and Mental Retardation limited to Females	EFMR
Epilepsy Multiplatform Variant Prediction	EpiMVP
Epilepsy of Infancy with Migrating Focal Seizures	EIMFS
Epileptic (Infantile) Spasms	ES
Epileptic Encephalopathies	EE
European Medicines Agency	EMA
Exome Sequencing	ES
Exome Sequencing	ES
Familial Febrile Seizures	FFS
Febrile Seizures	FS
Febrile Seizures plus	FS+
Fluorescent In Situ Hybridisation	FISH
Focal Cortical Dysplasia	FCD
Food and Drug Administration	FDA
Gain of Function	GoF
Generalised Genetic Epilepsy	GGE
Genetic Epilepsy with Febrile Seizures plus	GEFS+
Genome Sequencing	GS
Genome-Wide Association Studies	GWAS
Global Developmental Delay	GDD
Heterozygous	het
High Income Countries	HICs
Homozygous	homo
Human Heredity and Health in Africa project	H3Africa

List of Abbreviations cont.

Induced Pluripotent Stem Cells	iPSCs
Intellectual Disability	ID
International League Against Epilepsy	ILAE
Interquartile Range	IQR
Intragenic Complementation	IGC
Ketogenic Diet	KD
Lennox-Gastaut Syndrome	LGS
Lesional Focal Epilepsy	LFE
Long-read Sequencing	LRS
Loss of Function	LoF
Low and Middle Income Countires	LMICs
Magnetic Resonance Imaging	MRI
Malformations of Cortical Development	MCD
Multi-disciplinary Team	MDT
Multiple Ligase-dependent Probe Amplification	MLPA
National Health Laboratory Service	NHLS
Neurodevelopmental Disorders	NDD
Next Generation Sequencing	NGS
Non-communicable Disease	NCD
Pathogenic	P
Pathogenic/likely pathogenic	P/LP
Polygenic Risk Score	PRS
Precision Medicine	PM
probability of Loss of function Intolerance	pLI
Pyridoxine-Dependent Epilepsy	PDE
Randomized-Controlled Trial	RCT
Red Cross War Memorial Children's Hospital	RCWMCH
single molecule Molecular Inversion Probe	smMIP
Single/short Nucleotide Variants	SNV
Sodium Channel Blockers	SCBs
South Africa/n	SA
South African Medical Research Council	SA MRC
Spinal Muscular Dystrophy	SMA
Status Epilepticus	SE
Subependymal Giant Cell Astrocytoma	SEGA
Sub-Saharan African	SSA
Sudden Unexpected Death in Epilepsy	SUDEP
Think-Genetics decision tree	TG decision tree
Traditional Healer	TH
Traumatic Brain Injury	TBI
Tuberous Sclerosis Complex	TSC
Turn-Around-Time	TAT
University of Cape Town	UCT
Variant/s of Uncertain Significance	VUS
X-linked	XL
X-linked recessive	XLR
X-linked recessive	XLD

ABSTRACT

Background

Sub-Saharan Africa bears the highest burden of epilepsy worldwide. A proportion is presumed to be genetic, but this aetiology is buried under the burden of infections and perinatal insults, in a setting of limited awareness and few options for testing. Children with developmental and epileptic encephalopathies (DEEs), are most severely affected by this diagnostic gap, as the rate of actionable findings is highest in DEE-associated genes. This research study investigated the genetic architecture of epilepsy in South African (SA) children clinically diagnosed with DEE, highlighting the clinical utility of informative genetic findings and relevance to precision medicine for DEEs in a resource-constrained setting.

Methods

A group of 234 genetically naïve SA children with drug-resistant epilepsy and a diagnosis or suspicion of DEE, were recruited between 2016 and 2019. All probands were genetically tested using a DEE gene panel of 71 genes. Of the panel-negative probands, 78 were tested with chromosomal microarray and 20 proband/parent trios underwent exome sequencing. Statistical comparison of electroclinical features in children with and without candidate variants was performed to identify characteristics most likely predictive of a positive genetic finding.

Results

Pathogenic/likely pathogenic (P/LP) variants were identified in 41/234(17.5%)* probands. Of these, 29/234(12.4%)* were sequence variants in epilepsy-associated genes and 12/234(5.1%)* were genomic copy number variants (CNVs). Sixteen variants of uncertain significance (VUS) were detected in 12 patients. Of the 41 children with P/LP variants, 26/234(11%) had variants supporting precision therapy. Multivariate regression modelling highlighted neonatal or infantile-onset seizures with movement abnormalities and attention difficulties as predictive of a positive genetic finding. This, coupled with an emphasis on precision medicine outcomes, was used to propose the pragmatic “Think-Genetics” decision tree for early recognition of a possible genetic aetiology, pragmatic testing and multidisciplinary consultation.

Conclusion

The findings presented here emphasise the relevance of an early genetic diagnosis in DEEs and highlight the importance of access to genetic testing. The “Think-Genetics” strategy was designed for early recognition, appropriate interim management and genetic testing for DEEs in resource-constrained settings. The outcomes of this study emphasise the pressing need for augmentation of the local genetic laboratory services, to incorporate gene panels and exome sequencing.

*These percentages were rounded off to whole numbers in the published articles included in this thesis (i.e., rounded off to 18%, 12% and 5%, respectively).

1. INTRODUCTION

International research over the past two decades, empowered by next generation technologies, has uncovered more than 700 genes implicated in the pathogenesis of epilepsy. Of these, over 100 have been associated with the severe DEE phenotypes, and many have significant therapeutic and prognostic implications. Most of this gene discovery, however, was achieved through study of North American and European patient populations, with little - if any – published data available on the genetic underpinnings of epilepsy in African patients, and virtually no genetic epilepsy research conducted in Africa. This dearth of genetic knowledge and lack of diagnostic genetic testing puts patients with DEE in Africa at a significant disadvantage in terms of equitable access to precision treatment. The work described here, forms a foundation for further genetic epilepsy research on the African continent which, owing to the vast genetic variation of its populations, is likely to yield significant and internationally relevant insights. It also provides the groundwork for establishing a local genetic testing service for epilepsy, thus helping to bridge the existing diagnostic and therapeutic gap for people living with epilepsy in Africa.

1.1 Thesis Outline

This dissertation includes four publications describing the background and outcomes of this research study. The publications have been incorporated as text boxes using the font in the thesis (Arial 10) but preserving the article content as well as the respective journal's prescribed format and referencing style. The thesis comprises of six chapters, each addressing the following key aspects:

- The introductory Chapter 1 (Introduction) incorporates the Editorial published in January 2021 in *The South African Medical Journal* (SAMJ), describing the rationale for the study and the context within which this research was undertaken. This chapter also lists the study aims and objectives.

- Chapter 2 (Literature Review) is an overview of international research and current knowledge on the genetic architecture of epilepsy, its clinical application and relevance to personalised medicine. The chapter incorporates the peer-reviewed article published in May 2018 in *Frontiers in Neurology*, which addresses the challenges and opportunities for genetic epilepsy research in Africa The paper highlights the importance of lobbying for resources to build local research and clinical capacity and reduce the widening diagnostic and treatment gap for epilepsy in Africa.

- The peer-reviewed article published in November 2018 in *Seizure - European Journal of Epilepsy,* forms Chapter 3 (Pilot Study) and describes the main study pilot, investigating the genetic aetiology and clinical characteristics of 22 patients clinically diagnosed with Dravet Syndrome (DS), recruited from the Epilepsy Clinic at the Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town.

- The peer-reviewed article currently in press for publication in *Genetics in Medicine*, forms Chapter 4 (Main Study) and describes the genetic and clinical findings of the full study cohort of 234 children diagnosed with DEE, managed through the Epilepsy Clinic of the RCWMCH in Cape Town. In addition to the genetic analyses with targeted NGS panels, exome sequencing (ES) and chromosomal microarray (CMA), the

descriptive component of the study explores possible associations between selected clinical features and an identified genetic aetiology. The overall genetic and statistical outcomes were used to suggest a decision tree enabling early triage of newly presenting patients with possible DEE, for correct intervention and ideally, genetic testing. This proposed evidence-based protocol includes a genetic testing strategy for epilepsy in Low and Middle Income Countries (LMICs) and is suitable for testing in future validation studies.

- Chapter 5 (Discussion and Conclusions) incorporates the discussion of overall findings, the study strengths and limitations, further directions and conclusions. It describes the potential overall impact of the work presented in this thesis on the local clinical and laboratory protocols for paediatric epilepsy in SA. It emphasises the importance of translating the work done here, and expanding genetic epilepsy research for further benefit of people living with epilepsy in Africa.

- Finally, Chapter 6 contains the Supplemental Information of the main study article in Chapter 4 and includes Tables, Figures and information of interest, which could not be incorporated into the main article but contain important details of the outputs of this study. The format of the Supplementary Information (order of listing, numbering conventions etc.) is the same as that of the published article.

1.2 Study Rationale

The rationale behind this research into the genetic underpinnings of epilepsy in SA patients was described in an Editorial published in January 2021, in the South African Medical Journal. The editorial also mentions the pilot study of this project, which was published prior to this Editorial and forms Chapter 3 of this thesis.

Journal: South African Medical Journal (SAMJ)

Title: ***Importance of Genetic Diagnosis in the Management of Early-onset Epilepsies.***

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Epilepsy in Africa.

Sub-Saharan Africa (SSA) carries the greatest burden of epilepsy in the world, strongly linked to the high incidence of central nervous system infections, perinatal insults and traumatic brain injury[1]. Almost 60% of people with epilepsy in SSA do not receive medication and only about a third of those who do are appropriately managed[2]. The substantial risk of premature mortality associated with epilepsy in Africa (22.2 to 45.1 per 1000[3]) is strongly linked to poor seizure control. Reduced access to education, employment opportunities, as well as the social stigma attached to epilepsy in some communities, all place a burden on the individual and the family. The period of infancy carries the highest incidence of epilepsy[4], with some of the worst immediate and long-term sequelae, affecting all areas of the infants' and their carers' lives. Early diagnosis and correct management are critical in mitigating the detrimental effects of uncontrolled seizures on the maturing brain[5].

Most epilepsies previously termed 'idiopathic' (i.e., without a clear acquired cause) have a genetic basis[6]. The advent of next generation sequencing (NGS) created a massive surge in epilepsy gene discovery, revealing a previously unappreciated genetic and phenotypic heterogeneity. Over 350 genes have been associated with epilepsy to date[7] with a remarkably high frequency of *de novo* variants, especially among the developmental and epileptic encephalopathies (DEEs)[8]. Large-scale studies continue to expand and refine the phenotypic spectrum of known epilepsy-associated genes, with certain clinical features linked to variants in specific genes[9]. Examples include movement disorders and head stereotypies in *STXBP1*-related DEE[10], and clustered focal seizures restricted to females with *PCDH19* variants[11]. The genetic and phenotypic heterogeneity of epilepsy is exemplified by the *SCN1A* seizure disorders, where *SCN1A* variants may cause a severe, drug-resistant DEE (Dravet syndrome(DS))[12] in some patients, or milder disease in others (e.g., genetic epilepsy with febrile seizures plus (GEFS+))[13]. The precise determinants of this heterogeneity are still unknown but somatic mosaicism and functional effects of specific variants are known to play a role[14]. Importantly, knowledge of the causative mutation may guide the choice of treatment. Examples of precision therapies include the ketogenic diet for glucose transporter deficiency (*SLC2A1*), phenytoin or high-dose carbamazepine for *SCN2A* and *SCN8A*, and avoidance of sodium channel blockers in *SCN1A*-related epilepsy[15]. Genetic testing for epilepsy is now firmly embedded in the diagnostic setting of high-income countries (HICs), particularly for the DEEs, where the rate of informative findings is highest, with a demonstrable utility and cost benefit[16,17].

A recent global burden of disease (GBD) report ranked 'idiopathic epilepsy' (epilepsy of genetic origin or without a definite structural, metabolic, infective, or immune cause) as the second most common neurological disorder in southern-SSA[18]. Yet, little is known about the genetic architecture of epilepsy in SSA and no genetic testing is available locally[19]. Whilst local research may uncover new genes and variants, it is likely that the genetic aetiology of *de novo* epilepsies in Africa is similar to the HIC's. Therefore, research and translation in this instance should happen almost simultaneously. Unfortunately, the expense of genomic analysis is a major limitation, as the anticipated reduction in NGS cost has not materialised tangibly in Africa. Suppliers base price negotiations on projected throughput, which is difficult in a setting of limited budget for genetic services, particularly in the state sector overwhelmed by the burden of infectious

diseases. Paradoxically, it is often cheaper to refer NGS analyses abroad than to test locally, which may present an economical solution initially but does not serve to build local capacity. The shortage of suitably qualified workforce could be remedied by investment in training and creative use of the existing infrastructure but requires buy-in and financial support from the health authorities.

Opportunities: Research and translation in SA

Initiatives such as the H3Africa, aim to address the deficits in the genomic knowledge and capacity in Africa. Major emphasis lies on developing "hubs" for research, bioinformatic networks and biorepositories across the continent[20]. The exceptional genetic diversity of the African populations carries a far greater power of discovery than the more homogenous populations that form the basis of current knowledge. For instance, a recent case-control study involving 900 African patients, revealed significant enrichment for rare variants in constrained genes in individuals with schizophrenia, with a modest effect size[21]. Even though schizophrenia may be more heterogenous, three times as many patients of European ancestry were required to show a similar effect size in a study of generalised genetic epilepsy[17]. Research in Africa may not only identify novel genes and mechanisms, but also influence development of new therapeutic agents for the benefit of patients everywhere.

With this in mind, the Division of Human Genetics at the University of Cape Town and the Paediatric Neurology team at the Red Cross War Memorial Children's Hospital (RCWMCH) in collaboration with the Ken and Ruth Davee Department of Neurology at Northwestern University in Chicago have initiated research into the genetic causes of epilepsies in SA children. The rationale for this ongoing work combines the need to build knowledge on epilepsy genetics in SA and create a basis for diagnostic testing. Most of the participants are children with DEE, attending the Epilepsy Clinic at the RCWMCH. The USA collaboration lends valuable access to expertise in epilepsy genetics and NGS experimental design, helping to build capacity that is still centred in Africa. The project was successfully piloted with a subgroup of patients diagnosed with possible DS. The outcome not only helped to confirm or exclude DS, but also highlighted the onset of recurrent, prolonged febrile seizures before the age of six months as a simple diagnostic criterion for possible DS, useful in the resource-constrained African setting[22]. Correlating genetic findings with the phenotypic and electroclinical information in the main study participants has helped to refine the diagnoses and treatment in some cases, and detection of rare, novel variants has ended the 'diagnostic odyssey' for others, confirming the utility of testing.

The world and SA, albeit less robustly, have entered the age of genomics and precision medicine. However, full participation in this transition for the benefit of African patients requires investment into high-throughput genomic skills and platforms.

Ethics

The epilepsy research referred to in this article was approved by the Human Research Ethics Committee at the UCT (HREC 232/2015; 767/2017; 357/2019).

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Disclosures

The authors declare no conflicts of interests.

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1.3 Aims and Objectives

Project Aim

To investigate and delineate the genetic background of early onset epilepsies in a cohort of patients seen at the Paediatric Neurology service of the RCWMCH in Cape Town, with the view to developing a genetic diagnostic service for DEEs.

Objective 1

To categorise and describe the clinical phenotypes of a cohort of over 200 unrelated SA paediatric patients with DEE, initially grouped into four main areas, based on their electroclinical data:

- Dravet syndrome (DS)
- Early infantile epileptic encephalopathy (EIEE)
- Epileptic (Infantile) Spasms
- Other DEEs, which do not fit into the above categories.

Objective 2

To sequence the coding region of the *SCN1A* gene in patients clinically diagnosed with DS (n= ~20), to determine the presence and type of disease-causing variants and to compare this to the published data from studies on North American and European DS cohorts. To confirm all candidate variants using an alternative method (e.g., Sanger sequencing) and establish possible disease-association using *in silico* functional prediction tools and family segregation analyses. To classify the variants according to the ACMG (American Collage of Medical Geneticists) recommendations (1).

Objective 3

To sequence the entire study cohort using a targeted next generation sequencing (NGS) gene panel of 71 genes implicated in early-onset epilepsy, and to compare the results to published studies on North American and European patients. To confirm all candidate variants with Sanger sequencing and establish possible disease-association using *in silico* functional prediction tools and family segregation analyses. To classify the variants according to the ACMG recommendations (1).

Objective 4

To perform CMA analysis on the panel-negative children for detection of disease-causing genomic CNVs. To confirm the findings with other methods [e.g., Multiple Ligase-dependent Probe Amplification (MLPA)] and perform segregation analyses where appropriate. To compare the pick-up rate and the specific findings to the published literature.

Objective 5

To perform ES on selected patient/parent trios of probands with no findings on the gene panel or CMA testing, for identification of possible novel DEE-associated genes or variants in genes not included in the NGS panel. To confirm all candidate variants with Sanger sequencing and establish possible disease-association using *in silico* functional prediction tools and family segregation analyses. To classify the variants according to the ACMG recommendations (1).

Objective 6

To assess the molecular, electroclinical and neuroimaging findings in the study population, for possible genotype-phenotype correlations and compare this data to previously published studies in other population groups (European/North American). To identify any local population-specific associations, which may assist in local patient management.

Objective 7

To use the project data to design an efficient and cost-effective genetic testing strategy for the DEEs, for introduction into the diagnostic service arena i.e., to establish a diagnostic genetic service, including retrospective cascade counselling, testing and follow up in the affected families.

2. LITERATURE REVIEW

2.1 The African Context

The wide diagnostic and treatment gap for people with epilepsy in Africa is attributed to the limited or lacking healthcare infrastructure, severe shortage of trained personnel, stigma associated with the disease and poor access to antiseizure medications (ASMs) (2,3). Included below, is the *Perspective* article published in May 2018, in *Frontiers in Neurology,* describing the rapidly advancing knowledge of the underpinnings of genetic epilepsies, how this knowledge is used to improve the care of people, especially children, living with epilepsy in high-income countries (HICs), and the relevance and potential to do the same in Africa. The article addresses the reasons behind the current lack of insights and clinical genetic testing for epilepsy on the continent and highlights the exciting opportunities for research and discovery within the under-investigated, genetically diverse African populations.

Journal: Frontiers in Neurology

Title: ***Clinical Application of Epilepsy Genetics in Africa: Is Now the Time?***

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ABSTRACT

Over 80% of people with epilepsy live in low-to-middle-income countries, where epilepsy is often undiagnosed and untreated due to limited resources and poor infrastructure. In Africa, the burden of epilepsy is exacerbated by increased risk factors such as central nervous system infections, perinatal insults, and traumatic brain injury. Despite the high incidence of these aetiologies, the cause of epilepsy in over 60% of African children is unknown, suggesting a possible genetic origin. Large-scale genetic and genomic research in Europe and North America has revealed new genes and variants underlying disease in a range of epilepsy phenotypes. The relevance of this knowledge to patient care is especially evident among infants with early-onset epilepsies, where early genetic testing can confirm the diagnosis and direct treatment, potentially improving prognosis and quality of life. In Africa, however, genetic epilepsies are among the most under-

investigated neurological disorders, and little knowledge currently exists on the genetics of epilepsy among African patients. The increased diversity on the continent may yield unique, important epilepsy-associated genotypes, currently absent from the North American or European diagnostic testing protocols. In this review, we propose that there is strong justification for developing the capacity to offer genetic testing for children with epilepsy in Africa, informed mostly by the existing counselling and interventional needs. Initial simple protocols involving well-recognized epilepsy genes will not only help patients but will give rise to further clinically relevant research, thus increasing knowledge and capacity.

INTRODUCTION

Epilepsy affects approximately 70 million people globally. Of these, over 80% live in low-to-middle-income countries (LMICs) (1), where epilepsy is under-diagnosed and often untreated (2). The underlying reasons range from poorly resourced healthcare systems to the social stigma of epilepsy and reluctance to seek treatment. The high prevalence of epilepsy, particularly in sub-Saharan Africa (SSA) coexists with increased risk factors, especially central nervous system infections, perinatal insults, and traumatic brain injury (3). Epilepsy due to genetic, immune, metabolic, or structural causes is rarely recognized, and its burden is virtually unknown (Figure 1) (3).

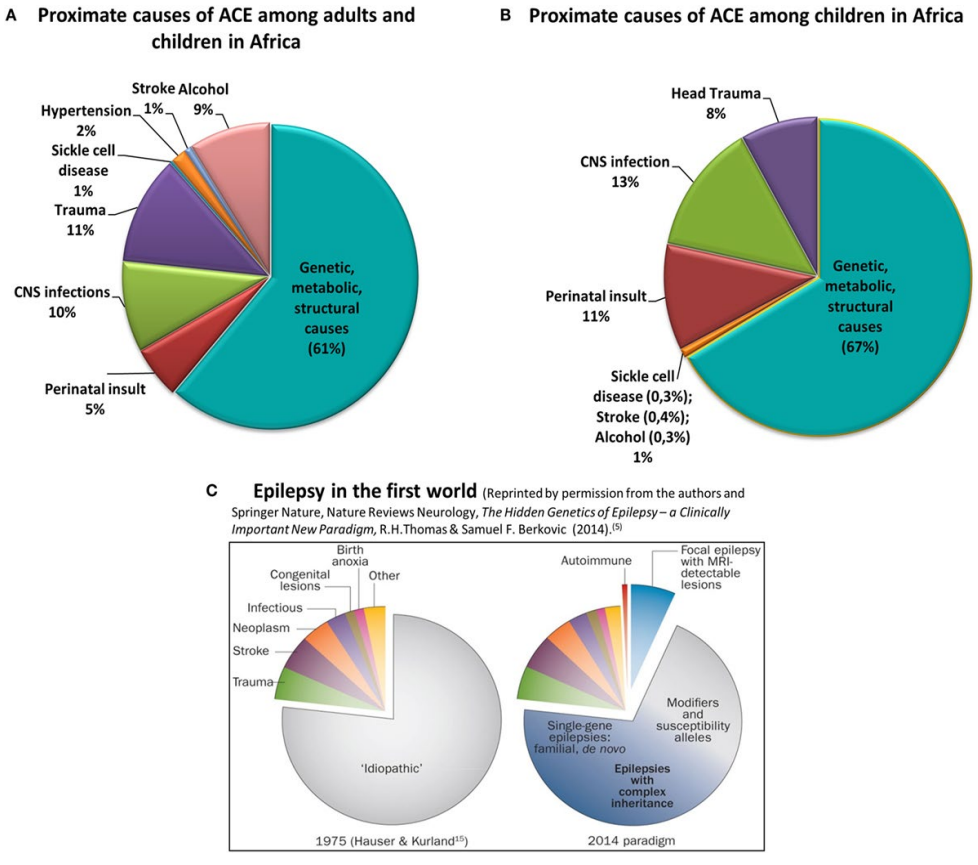

Figure 1. **Proximate causes of active convulsive epilepsy (ACE) in Africa according to currently available knowledge**. Charts (A,B) depict the distribution of proximate causes of ACE among all ages (A) and among children in Africa (B) [data obtained from Kariuki et al. (3) and personal communication with co-authors]. Chart (C) reflects the causes of all epilepsies in the first-world environment (5). Charts (A–C) clearly show the significantly higher burden of epilepsy due to central nervous system infections, trauma, and perinatal insult in Africa, particularly among children. Also emphasized is the absence of reliable knowledge on the burden of the genetic, metabolic, and structural causes of epilepsy in Africa.

The causal role of genetic variants in epilepsy is increasingly recognized. Over the past two decades, large-scale studies empowered by genomic technologies have shown that many epilepsies previously classified as “idiopathic” have a genetic basis (4, 5) (Figure 1). Effective investigation of genetically and phenotypically complex disorders such as epilepsy requires laboratory protocols incorporating next-generation sequencing (NGS) and chromosomal microarray, now routinely employed in the diagnostic centres of high-income countries (HICs). In Africa, however, resource allocation for genetic services is not prioritized, thus the necessary skills and equipment are lacking. Genetic epilepsy research is practically absent, resulting in few insights into the architecture of the disease in African populations. In this review, we examine the historical and current demographics of epilepsy and the medical diagnostic infrastructure in Africa. Within the context of global genetic research and its impact on personalized medicine, we argue that the time for epilepsy genetics in Africa is now and propose tangible actions to improve access to genomic technologies and diagnostic testing.

DISEASE BURDEN AND MANAGEMENT OF EPILEPSY IN AFRICA

The Global Burden of Diseases, Injuries and Risk Factors Study 2010, reported the burden of untreated severe epilepsy second only to HIV infection (6). Epidemiological studies over the past decade show that SSA carries the greatest prevalence and disability burden of epilepsy in the world, with the median prevalence estimated at 14.2 per 1,000 (IQR 8.0–33.2), more than double the prevalence in HICs [5.8 per 1,000 (2.7–12.4)] (3, 7). The true prevalence is likely to be higher, as many cases are not reported and seizures with fewer motor manifestations often go unrecognized (7, 8).

Almost 60% of people with epilepsy in SSA do not receive medication and only about 33% of those who do are appropriately managed (7). The likely reflection of this is the substantial risk of premature mortality in people with epilepsy in Africa, reported at rates ranging from 22.2 to 45.1 per 1,000 (9). Epilepsy also carries with it a significant “social disability” aspect, which is not reflected by the disability weight estimates. Reduced marriage prospects, less access to education and lower employment opportunities all place an additional burden on the individual and the family (7, 10). The “treatment gap” for epilepsy in Africa, defined as a percentage of people living with active untreated epilepsy is 47% in urban regions, compared with 73% in the rural areas, where prognosis and outcomes are poor (11). Treatment guidelines are usually created in well-resourced environments and require major adaptation to fit the African context (11). The recommended diagnostic tools and newer generation antiepileptic drugs (AEDs) are available only in the major tertiary centres of the state sector or in private practice (12). Typically, the correct diagnosis and treatment of early-life epilepsies in African children is achieved months or years after initial presentation and many die undiagnosed. The resulting financial burden placed on the healthcare system in these situations could be alleviated by early genetic diagnoses and timely intervention (13).

In South Africa, non-communicable diseases (NCDs) gained statistical prominence in the early 1990s, only to recede under the burden of the HIV/AIDS and TB pandemics (14). In 2015, however, the infant mortality rate dropped below 40/1,000 live births, signalling the need for resource allocation toward better service provision for NCDs (15). Recognition of the immediate and long-term value of genetic services is an imperative part of this transition.

EPILEPSY AS A GENETIC DISEASE

The major finding of epilepsy research in recent years is the high prevalence of de novo pathogenic variants, particularly well noted in developmental and epileptic encephalopathies (DEEs) (16–19). DEEs are characterized by pharmacoresistant seizures, severe electroencephalography (EEG) abnormalities and developmental delay (DD)/regression/intellectual disability. Approximately 40% of seizures with onset in the first 3 years of life will progress to DEE, and a substantial number of these are associated with variants in known epilepsy genes (20). While the majority of DEE patients carry de novo pathogenic changes, recent studies suggest that parental somatic mosaicism is present in up to 10% of the cases (21). This has important implications for genetic counselling and family planning, as the risk of recurrence in these families may be as high as 50% (21).

Although clinical and genetic heterogeneity is the hallmark of genetic epilepsy, certain genes and variants co-exist with characteristic clinical features. For instance, pathogenic *SCN1A* variants are identified in 80% of patients with Dravet syndrome (22). Movement disorders and head stereotypies are often seen with *STXBP1* variants (23) and clustered focal seizures with affective symptoms are seen in females with *PCDH19* variants (24). Awareness of such features is important in implementing cost-effective testing with a high yield of informative results. Furthermore, disease-causing genes in the severe forms of epilepsy are also implicated in a broader spectrum of epilepsy and associated neurodevelopmental disorders (25). Therefore, testing and therapeutic protocols targeting rare genetic epilepsies may also find application among the more common phenotypes (26).

GENETIC TESTING FOR EPILEPSY

The value of genetic testing in epilepsy has been debated and depends on the phenotype and reason for testing. Presently, testing for early-onset epilepsies appears to yield the most informative results, as recently emphasized in a report by Berg and colleagues. In a group of 327 children with seizure onset before the third year of life who underwent some form of genetic testing, pathogenic variants were identified in 132 (40.4%) of the cases (27). While clinical whole-exome sequencing is gaining popularity as a first tier assay for genetically heterogeneous disorders, the targeted approach is mostly still favoured in the clinical setting. NGS panels have increased specificity, greater depth of coverage (better sensitivity), less exonic dropout, and fewer issues relating to incidental findings (13, 28). The main disadvantage is missing a pathogenic change in a gene not included in the panel. Chromosomal microarray analysis for genomic copy number variants (CNVs) is indicated in children with seizures accompanying DD/intellectual disability, as there is evidence that up to 10% of these patients have disease-associated CNVs (29).

Genomic technologies are expensive to establish and maintain, and require technical and bioinformatic expertise. In South Africa, the established infrastructure for genetic services includes laboratories and clinical services. Regular specialist clinics and outreach initiatives strive to increase awareness and deliver services both locally and to the neighbouring, more resource-limited SSA countries, highlighting the need to build capacity across the continent. Despite the challenges of diagnosis and treatment and even in absence of population-specific data, there is sufficient justification to support availability of early screening protocols for specific epilepsy phenotypes. Neuroimaging, ideally magnetic resonance imaging (MRI), and EEG

remain essential investigations in the diagnosis of structural brain pathology. However, for many generalized epilepsies, MRI and EEG findings lack the specificity and consistency of an informative genetic test result. Most DEEs are genetic in origin, with an increasing number of clinical markers linked to specific genetic aetiologies (27). Access to neuroimaging and EEG in some LMICs is very limited, while DNA studies on saliva may become far more accessible at a similar cost. Therefore, resource allocation for genetic testing versus MRI should be prioritized on the basis of the clinical semiology, for early and targeted care (30).

The largely *de novo* aetiology of DEE shown in the HICs, should not be vastly different in Africa and while new, "African" epilepsy genes may emerge with future research, the DEE panels currently used in the HICs should also benefit African patients. Genetic testing may sometimes present a more direct and cheaper diagnostic tool than the traditional options, prevent the use of potentially seizure-exacerbating therapies (e.g., carbamazepine for *SCN1A*-associated DS) and further invasive and expensive investigations. These aspects are equally important to patients and families all over the world, and the benefits carry far-reaching health, psychological and socioeconomic consequences.

EPILEPSY RESEARCH IN AFRICA

The existing reports of neurogenetic research in Africa have shown that the genetic underpinnings of certain neurological phenotypes segregate almost exclusively in African populations. Examples include Huntington disease-like type 2 (31, 32), spinocerebellar ataxia type 7 (SCA7) (33, 34) and *RYR1*-related centronuclear myopathy (35). However, published epilepsy research emanating from the continent focuses on the disease epidemiology, aetiology and management (3, 8, 36–40), and little is known about the genetic causes in African patients. More research is needed to identify the role of presently unknown genetic causes and risk factors for epilepsy in people of African ancestry.

Consanguinity and febrile illness feature strongly as risk factors for epilepsy and genetic disease in general. Both occur frequently in African populations, presenting an excellent focus for research which has not been fully explored. Only a few North African studies of ion channel genes in febrile seizure phenotypes (41, 42) and small cohorts of familial epilepsies in consanguineous families have been published (43–45). For cultural reasons, consanguineous unions are more common in North African countries, compared with the rest of the continent (46). Thus, the obvious implications for recessive disease are less relevant to populations in SSA where consanguinity is uncommon, emphasizing the need for population-specific translatable research, genetic counselling and education. Furthermore, research within the context of the increased genetic diversity in Africa may provide additional insights into the underpinnings of familial epilepsies. The large African sibships and increased twinning in some regions (47, 48) offer a valuable opportunity for genetic studies and a better understanding of brain development, potentially opening the way to the discovery of new therapeutic agents.

The highly variable response to AEDs is complicated by the African diversity and the challenges of managing comorbidities and medications (e.g., malaria, TB, HIV, and schistosomiasis) (49). Many AEDs are substrates for the Cytochrome P450 (CYP) enzymes, which are important determinants of response to most drugs

prescribed today. Polymorphisms in the encoding genes are linked to altered levels of activity and adverse drug reactions (50). As an example, individuals carrying the CYP2C9*2 (rs1799853) and CYP2C9*3 [rs1057910(C)] polymorphisms metabolize phenytoin at a markedly slower rate and a higher risk of concentration-dependent neurotoxicity than individuals homozygous for the wild-type allele [CYP2C9*1; rs1057910(A)] (50, 51). However, compared with the European and Asian populations, allele frequencies of these variants among Africans are much lower, negating any real public health applicability in Africa (52, 53). The frequencies of known CYP enzymes show a considerably greater variability across Africa, with a few polymorphisms reported only in African populations. Most research to date focuses on allele frequency distributions of known polymorphisms, and there is a need for discovery of new markers and profiling that is relevant in Africa (54). Adding to this complexity is the widespread use of herbal products, with possible herb–drug interactions which may affect efficacy and toxicity profiles of pharmaceutical drugs (55). Undoubtedly, discovery of new genetic markers of drug response will be of global value, as the ancestral origin of the human population is represented in the African genomes.

While genomic research has been powering ahead in the rest of the world for almost two decades, Africa remains far behind. Insights into the genetic diversity in Africa gained through the Human HapMap, and the 1000 Genomes Project was limited to the Yoruba and Esan (Nigeria), Mende (Sierra Leone), and the Luhya and Masai (Kenya) populations, leaving much of the African continent unexplored (56, 57). If this lag continues, the potential health and economic benefits emanating from genomic science may elude an entire continent (58). Initiatives such as the Human Heredity and Health in Africa project (H3Africa) and the African Genome Variation project (AGV) are designed to urgently address this gap in knowledge and capacity (58, 59), with an emphasis on “Afrocentric” genomic research, biorepositories, and bioinformatic networks (58). This should carry the benefits of improved variant databases, as well as development of preventative and targeted treatments in the age of precision medicine. The increasing burden of NCDs in SSA makes a strong case for more financial and intellectual investment into genetic research in Africa and into translating the outcomes into medical practice.

NEEDS AND CHALLENGES

Many clinicians in Africa are not trained to recognize a possible genetic epilepsy and focus mainly on prescribing treatment. Signs and symptoms are blurred by the layering effects of untreated seizures and multiple insults of birth trauma, co-infections, nutritional insults, and socioeconomic issues. In SSA, acute symptomatic and febrile seizures are frequently assumed to be due to malaria, limiting the search for other causes. Little consideration is given to the increased subsequent risk for epilepsy, much less the possible genetic basis for this risk (60). Often, a genetic aetiology is considered only when a second affected child is born. In the setting where families are already struggling to cope with the complex health care of the first affected child, this can become untenable. Efficient management of epilepsy is particularly important in childhood because of the detrimental effects of uncontrolled seizures on the developing brain (1, 61, 62). The prevailing social stigma of epilepsy often labels these children as infectious, mentally ill or spiritually “possessed” (36, 63). It is important to recognize the potential value of community leaders, elders, and traditional healers (THs), in addressing these issues.

Many Africans, particularly in the rural setting, are inclined to seek treatment from a TH rather than a "Western-style" medical doctor. Traditional medicine is seen as more relevant to the African ways of living and, most importantly, it is more accessible. The person-to-neurologist ratio in SSA is up to 5,099,908 persons per neurologist, depending on the region. In contrast, the person-to-TH ratio in the SSA is approximately 1:200 (63). The cost of traditional medicines is not necessarily lower than the more affordable AEDs (e.g., phenobarbital), but a consultation with a TH can be considerably cheaper and is viewed as better value for money (64). A TH spends more time with the patient, counsels the whole family and often accepts non-monetary forms of payment, such as home produce or livestock. It is therefore imperative that the educational programs include THs, who can significantly contribute toward removing the stigma of epilepsy and facilitating treatment. There are also social and cultural beliefs attached to genetics and genetic disease. Many religious African communities instil a sense of acceptance and view genetic testing as interference with "god's will." This is not necessarily linked to a level of education but may stem from a lack of understanding of the choices available (65). Therefore, information and counselling is imperative for patients, families as well as THs. An additional challenge in SSA with its high morbidity and mortality due to HIV/TB/malaria and migrant labour practices is the phenomenon of "orphan households" (66). The high prevalence of households with single or no biological parents renders genetic testing of family trios impossible, complicating research and diagnostic testing protocols. Biological non-paternity is another issue which must be considered, carrying with it significant ethical implications.

To the best of our knowledge, no genetic testing for epilepsy is presently available in SSA. The available genetic testing is generally limited to monogenic diseases and specific, common pathogenic variants. NGS is not routinely performed, though it may sometimes be outsourced through private laboratories for those who can afford it, as even medical insurance does not always cover the cost. There is also a need for population-based databases and repositories of genomic variants, for correct variant interpretation in the African context.

Therefore, the question that begs asking is whether there is justification in the setting of such obstacles and limited resources, for apparently elite medicine. In our opinion, the answer is "yes" but implementation requires a political and financial engagement from health authorities. Outsourcing of testing to service providers in the HICs presents an economical solution initially, but does not serve to build skills and capacity in Africa. In a middle-income country like South Africa, creating local capacity can be achieved relatively easily with creative use of available infrastructure and an investment in training and human resources. NGS costs are dropping, and manufacturers are focusing on solutions for better scalability and cost-effective analysis of smaller sample batches within clinically relevant turn-around-times. Service-level agreements with local technical service providers (e.g., sequencing facilities affiliated to universities or commercial companies) are being explored.

CONCLUSION AND FUTURE DIRECTIONS

Reducing the epilepsy treatment gap in Africa requires improved access to multidisciplinary care (67). The clinical utility of genetic testing in epilepsy presents a compelling case and an opportunity to bring NGS technology into diagnostic laboratories in Africa. It is time to for practical solutions with tangible outputs:

- Training for healthcare professionals in primary healthcare, to create awareness of genetic epilepsies and key clinical identifiers of patients most likely to benefit from testing.
- Education initiatives addressing the misconceptions and prejudices toward epilepsy, genetic disease, and testing aimed at patients, families, community leaders and THs. Here, genetic counsellors have an important role to play. SA is the only African country offering Masters-level training for genetic counsellors who struggle to find employment post qualification. There is a need for job creation and increased capacity.
- Establishing referral systems between the THs and medical clinics, facilitating access to AEDs, psychosocial support, and genetic counselling.
- It is crucial that the knowledge gained and resources created through projects such as H3Africa and the AGV are accessible to the diagnostic laboratories.
- Initially, small physician–researcher collaborations are likely to drive epilepsy genetic research in Africa. Genetic testing of over 200 patients with DEE is currently underway at the University of Cape Town, in collaboration with the Northwestern University in Chicago. It is hoped that the project will create a basis for a variant database, give rise to a genetic service for epilepsy, and act as a springboard for more epilepsy research in SA and more broadly on the African continent.

ETHICS STATEMENTS

This article forms a part of the justification for the genetic epilepsy research referred to in the text, currently underway at University of Cape Town, in collaboration with Northwestern University in Chicago. The research study was granted ethical approval by the Human Research Ethics Committee of the University of Cape Town, in accordance with the Declaration of Helsinki (HREC REF 232/2015).

AUTHOR CONTRIBUTIONS

AE: article design, drafting, collation of information, and critical revision; GC and AP: article design and critical revision; RR: critical revision; SK and CN: contribution of epidemiological information and critical revision; JW: article conception, critical revision, and professional communication.

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2.2. Current Landscape of the Rare Genetic Epilepsies

2.2.1. Terms and Definitions

The International League Against Epilepsy (ILAE) defines a seizure as a transient occurrence of signs and symptoms resulting from abnormal, excessive or synchronous neuronal activity in the brain. Seizures are initially defined by their onset, which can be focal (arising in specific region of the brain), generalized (arising in both cerebral hemispheres) or of unknown onset (4).

Epilepsy is a condition associated with an enduring predisposition to seizures, defined as a disease of the brain resulting from any of the following conditions (5):
1) at least two unprovoked (or reflex) seizures occurring more than 24hrs apart or
2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years, or
3) having a diagnosis of an epilepsy syndrome.

The diagnosis of epilepsy can be refined at three levels, depending on the available information and access to testing: (1) seizure type, (2) epilepsy type and (3) epilepsy syndrome. In addition to the seizure type, this ILAE-approved diagnostic framework, takes into account the age at seizure onset, electroencephalography (EEG) and magnetic resonance imaging (MRI) features (Figure 1) (5). Epilepsy syndromes are often associated with specific seizure types, EEG signatures, comorbidities and age-related manifestations (6). The underlying cause may be of genetic or acquired origin involving metabolic, immune or inflammatory processes, or structural brain abnormalities resulting from developmental malformations or injury through infections, pre and perinatal trauma or hypoxic-ischemic insults (5). The quality of life and health in people living with epilepsy is directly linked to the level of seizure control, which is successful in many individuals, allowing them to live essentially normal lives. This, however, is not the case in individuals with the developmental and epileptic encephalopathies (DEEs), who have multiple comorbidities in addition to drug-resistant seizures (7).

The term “developmental and epileptic encephalopathies” was introduced with the 2017 revision of the ILAE classification to recognise the two-fold causation of the developmental impairment, where 1) the underlying genetic aetiology is responsible for the developmental encephalopathy independent of seizures and 2) the ongoing, frequent seizures cause an epileptic encephalopathy, which also adversely affect development, resulting in developmental slowing or neuroregression (8). This may occur at seizure onset, exacerbation or following status epilepticus (SE). Seizure control significantly improves the overall development and outcomes, although a baseline level of developmental impairment persists, owing to the underlying disease mechanisms. In addition to drug-resistant seizures, developmental delay (DD) and intellectual disability (ID), DEE patients typically have a wide range of comorbidities including psychiatric problems [autism spectrum disorder (ASD)], depression and psychosis, movement abnormalities, speech, sleep, gastrointestinal difficulties and an increased mortality rate. Epilepsy in most DEEs has neonatal or infantile onset.

Following the discovery of the *CHRNA4* gene implicated in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in 1994 (9), gene discovery in epilepsy is has been described as having taken place in three distinct phases: (1) the pioneer era of laborious gene discovery mainly involving the channelopathies and familial epilepsy syndromes, (2) a relatively dormant period of mostly negative genome-wide association studies (GWAS), and (3) the era of genomic sequencing and rapid gene discovery, especially in the severe, early-onset epilepsies (10).

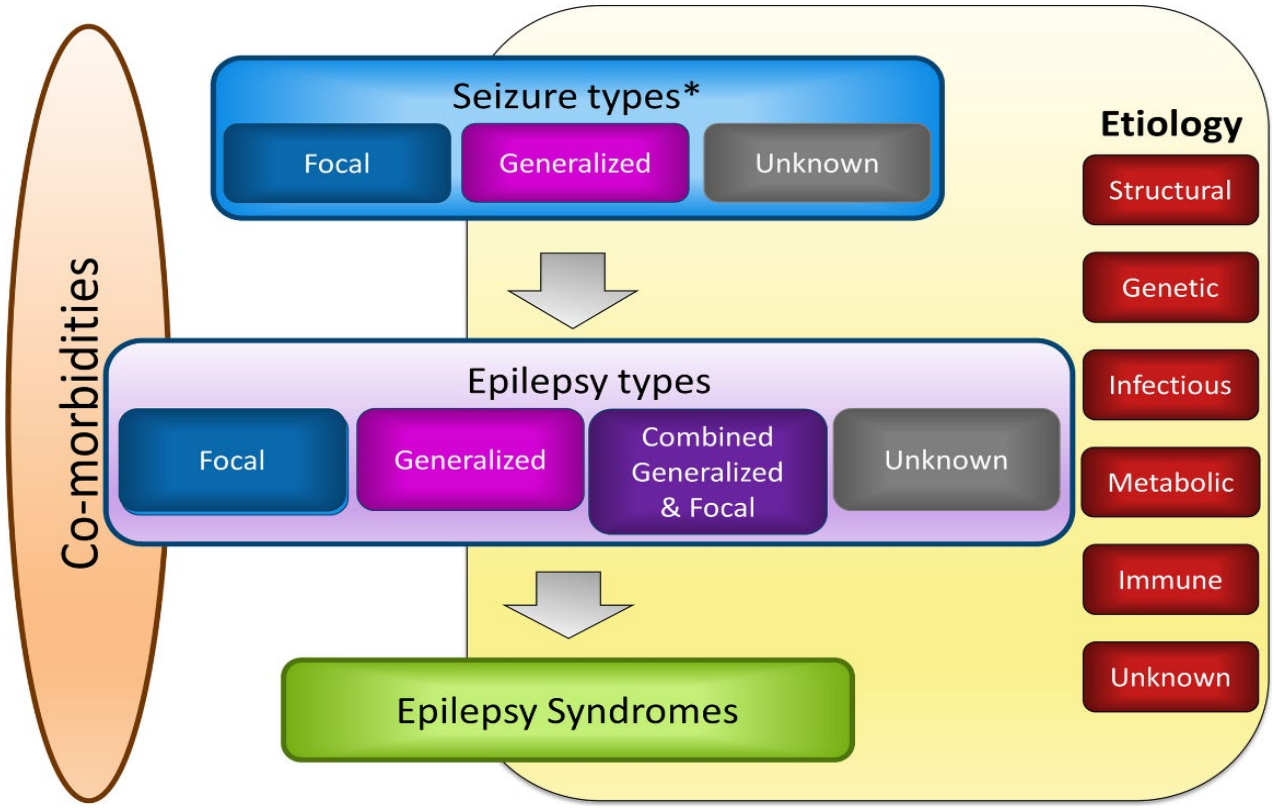

Figure 1. **Framework for classification of the epilepsies.** *Denotes onset of seizures. The ILAE Classification of the Epilepsies defined three diagnostic levels including (1) seizure type, (2) epilepsy type, and (3) epilepsy syndrome, emphasizing that aetiology and comorbidities must be considered at each level**.** Permission to re-use from reference (11), Schaffer et al., (2017), through RightsLink.

The success of gene discovery in epilepsy is attributed primarily to the technological advancements in DNA sequencing and genomic copy number detection, allowing for rapid, genome-wide analysis of multiple DNA targets (genes, exomes and genomes) in large cohorts of patients with epilepsy (12–14). This was aided by new bioinformatic capabilities enabling aggregation of large population datasets (15), as well as new data matching platforms, allowing investigators (as well as patients and families) to reach out to the international scientific and clinical community and connect with individuals or groups with similar interests or findings (16). Epilepsy gene discovery has been especially impactful for the DEEs, with over 100 implicated genes. Currently, a molecular diagnosis is obtainable for approximately 30-50% of the DEE patients, through routine clinical testing(17,18). Most cases of DEEs are caused by pathogenic *de novo* variants in dominant genes (19,20), though some are inherited from a mosaic parent (21–23). Many of the DEE-associated genes were discovered through testing of proband/parent trios and demonstrating *de novo* occurrence remains a major (though not the only) criterion in DEE-variant interpretation.

The DEE- genes encompass many areas of neuronal function, including regulation of synaptic transmission, cell growth, cell proliferation and chromatin remodelling, in addition to the ion channels and neurotransmitter receptors (Figure 2) (19,24–27). The underlying genetic aetiology has been shown to carry implications for treatment, realising the promise of precision medicine (PM) for many DEE patients (17). Rare genomic CNVs are another significant disease mechanism in epilepsy, known to underly disease in 4 - 8% of the DEE patients (28). CNVs at genomic hotspots such as 15q13.3, 15q11.2, 16p11.2, 16p13.11 and 22q11.2, have also been shown to confer risk for generalised genetic epilepsy (GGE) and lesional focal epilepsy (LFE) (29,30).

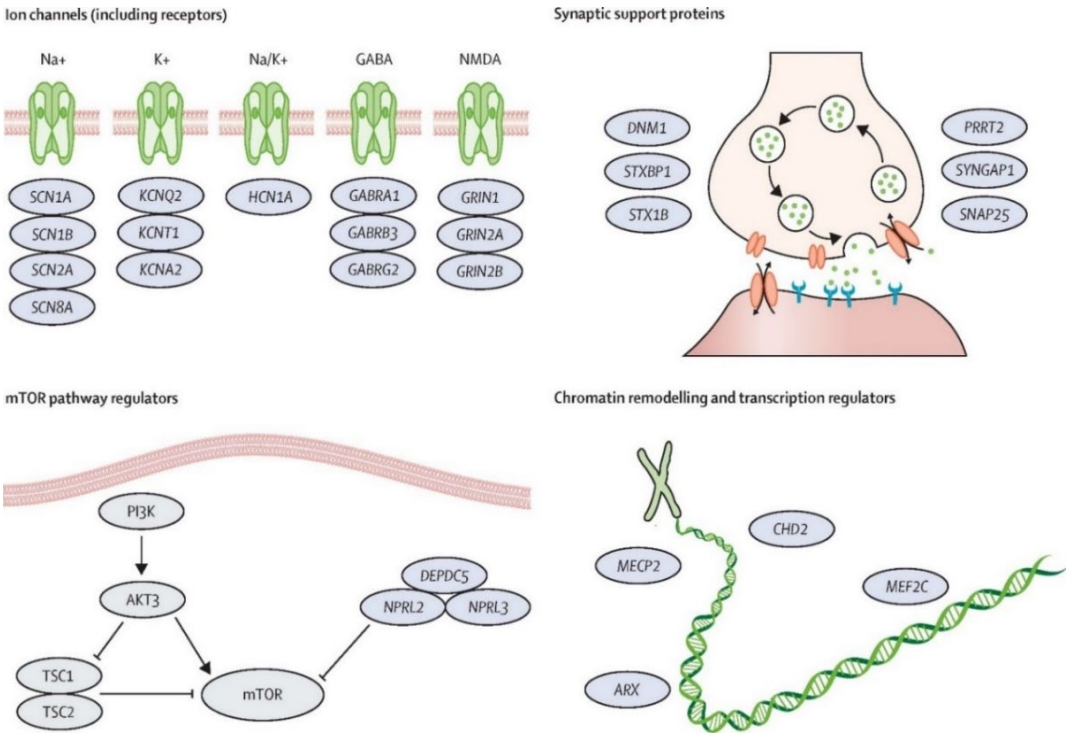

Figure 2. **Cellular functions and pathways implicated in epilepsy.**
The diversity of potential neurobiological mechanisms involving genes encoding proteins that regulate synaptic function, cell growth, cell proliferation, chromatin remodelling, and other cellular functions, in addition to ion channels and neurotransmitter receptors. Symbols for genes encoding the relevant proteins are shown in italics. mTOR=mechanistic target of rapamycin. Permission to re-use from reference (27) (Ellis et al., 2020) through RightsLink.

2.2.2. Clinical testing and variant interpretation

Genetic testing for epilepsy, particularly the DEEs, is now routinely available in the clinical genetic laboratories of HICs. Owing to the genetic and phenotypic heterogeneity of epilepsy and the DEEs, the recommended first-tier testing approach is multigenic or genome-wide (e.g., gene panels, ES, CMA), rather than targeted [e.g., single-gene screens, fluorescent in situ hybridisation (FISH)]. The recently published clinical testing guidelines for epilepsy recommend exome (ES) or genome sequencing (GS) incorporating CNV analysis into the bioinformatic pipeline (31). Targeted testing modalities such as Sanger sequencing, MLPA, FISH are mainly employed as confirmatory tests or for variant segregation analyses (Figure 3).

Data generated with high-throughput, multi-targeted testing includes hundreds of non-disease associated variants, presenting variant prioritisation and interpretation challenges. Establishing variant pathogenicity

requires rigorous evaluation of multiple lines of evidence, including allele frequencies in the relevant population, the frequency of a given variant type (e.g., missense or truncating) in a particular gene, segregation with phenotype within families and functional effects of the variant in *in vitro* and *in vivo* models. Specific criteria have been developed to aid and standardise the process of variant assessment for gene discovery, gene curation, and clinical diagnostic purposes (1,32). The limited available information on genetic variation in African populations, especially in Sub-Saharan Africa (SSA) is problematic for variant interpretation in patients of African descent, given the increased genetic diversity in Africa, compared to other populations (33). Initiatives such as the Human Heredity and Health in Africa project (H3Africa) and the African Genome Variation Project (AGV) aim at bridging the gaps in genomic knowledge and capacity (34,35). However, the data generated to date is not easily publicly accessible at this time.

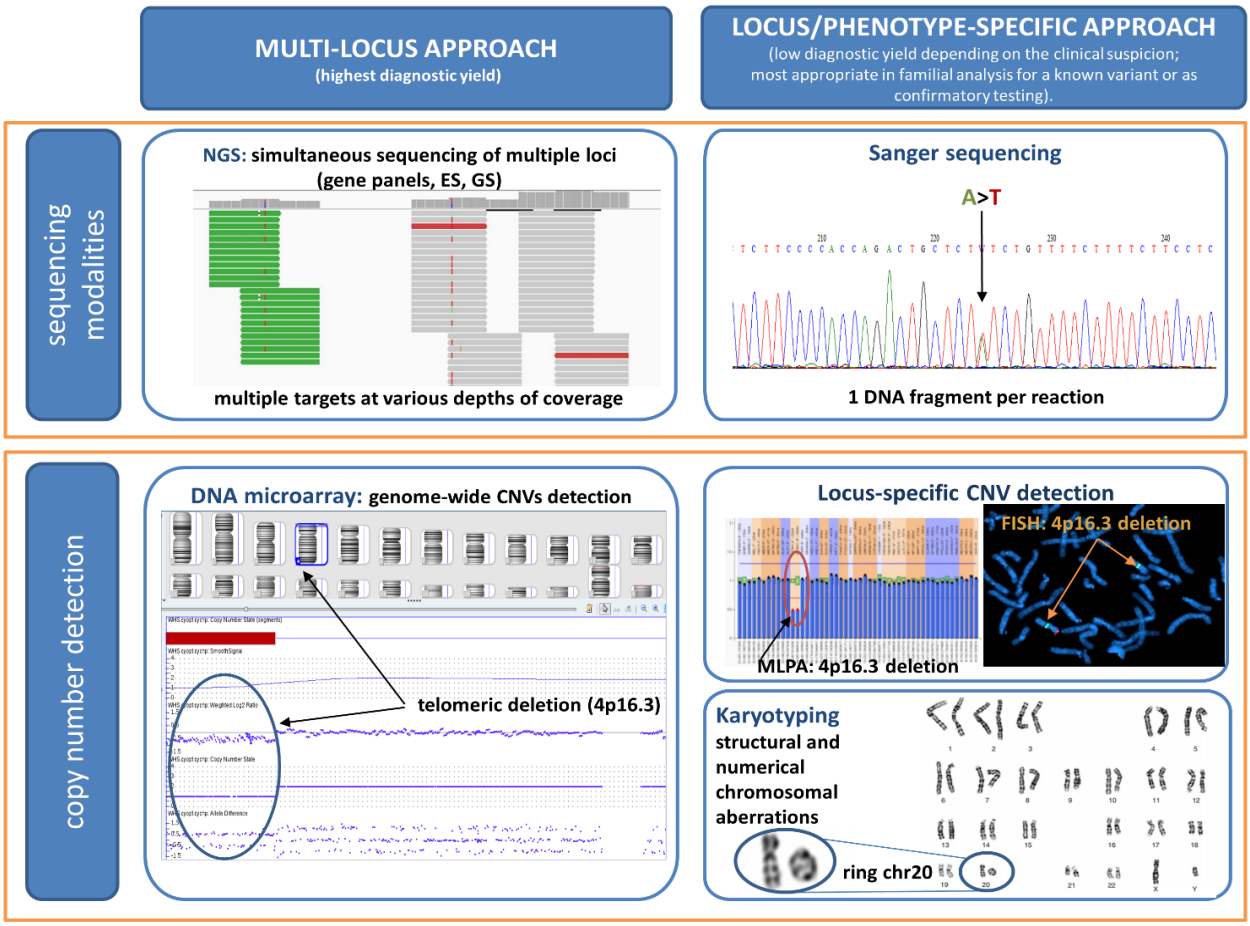

Figure 3. **Multi-locus and targeted testing modalities for sequence changes and copy number variations.** CNV = copy number variant; ES = exome sequencing; GS = genome sequencing; MLPA = multiple ligase-dependent probe amplification.

2.2.3. The Expanding Landscape of Genetic Epilepsies

Despite the successes in epilepsy gene discovery over the past decade and the emerging PM approaches, the genetic aetiology in approximately half of the DEE cases is still unknown, and the genetic landscape of the more common phenotypes, such as the non-acquired focal epilepsies (NAFE) or the GGEs is poorly understood. It is likely that the common epilepsies have an oligo- or polygenic architecture, with both common and rare variant contributions (36). Non-acquired focal epilepsies appear to be mainly associated with rare variants (germline

and somatic) with minimal contributions from common variants (37), whilst the GGEs have a stronger association with common variants (36), though the specific variants are not known. Some of these genetic contributions form part of the so-called missing heritability, which is currently challenging to investigate owing to (as outlined by Perucca et al., 2020):

a) lack of appropriate analytical methods,
b) the hypothesized large sample sizes necessary to accommodate the anticipated increased multiple-testing burden, and
c) the currently prohibitive cost of the multi-omics approach required to generate and interrogate data on methylation, histone modifications, transcriptomes etc., in large cohorts (36).

Further elucidation of the underlying causes and precision therapies for genetic epilepsies requires large-scale, collaborative efforts, involving diverse datasets and multi-omics approaches. The largest international epilepsy research initiative to date is the Epi25 Collaborative, aiming to sequence the exomes and genomes of 25 000 individuals with epilepsy (38) (http://epi-25.org/). In addition to new gene discovery, the goals of this initiative are to untangle some of the complexities of the common epilepsies and determine the significance of many genes and variants whose role in disease causation is currently uncertain. It should also refine the phenotypic spectra associated with specific genes and facilitate the design of clinical trials. Some of the recently gained insights into the causes, natural histories, therapeutic development and preclinical models are outlined below.

Genomic CNVs are a well-established cause of genetic epilepsies previously implicated in the DEEs, GGE and the focal epilepsies, with known genomic hot-spots and risk-loci (13,28,29,39,40). A recent genome-wide CNV meta-analysis investigated the burden and risk for epilepsy conferred by specific CNVs in different epilepsy subtypes in a cohort of 10 712 European patients with GGE, DEE, LFE or NAFE, and 6746 ancestry-matched controls (30). The findings revealed striking differences in CNV burden across epilepsy types and CNV categories, with the highest burden across all categories noted in individuals with GGE, followed by the DEEs. Both DEE and GGE patients showed a significant burden of deletions in genes intolerant to truncations, whilst the GGE patients showed enrichment for deletions at previously identified epilepsy hotspots, mainly 15q13.3 and 16p13.11. Compared to controls, an increased burden of 16p13.11 deletions was noted in patients with LFE. The large sample set and uniform CNV-calling pipeline enabled genome-wide CNV breakpoint association analysis. Out of seven previously established CNV associations, three achieved genome-wide significance in this study (15q11.2, 15q13.3 and 16p13.11) (29,39,41,42). The remaining four (1q21.1, 16p11.2, 16p12, and 22q11.2) only reached suggestive significance (P-value 5 0.05) in this dataset. These loci had also been previously associated with other neurodevelopmental phenotypes such as autism, psychiatric disorders and ID (43,44). Importantly, the established epilepsy-associated 15q13.3 deletion was shown to represent the strongest risk CNV for GGE across the genome. The investigators conclude that 1.5–3% of patients with the common epilepsy types carry epilepsy-associated CNVs which vary significantly across and within the epilepsy types. The investigators hypothesise that application of this collaborative framework to even larger datasets could potentially advance the discovery of loci and identification of critical genes and functional elements (30).

De novo variants are a known, major contributor to the DEEs (45). It is now recognised that **somatic mosaicism** (post-zygotic variants present in only a proportion of the cells in a body) represents a clinically relevant fine point

in the *de novo* paradigm for epilepsy. Investigators have shown that approximately 8% of the apparently *de novo* variants in DEEs are inherited from a mosaic parent, which has important implications for recurrence risk assessments and genetic counselling (21). Somatic mosaicism has also been suggested as a disease modifier in *SCN1A*-related epilepsy. A study of 128 patients previously diagnosed with *de novo SCN1A*-related phenotypes, identified mosaicism for the disease-causing variant in approximately 8% of the cases (22). On average, patients with mosaicism had milder phenotypes, suggesting that mosaicism may contributes to the phenotypic heterogeneity seen with many epilepsy genes. Detection of mosaicism requires deep sequencing of unique reads, which is a consideration for the diagnostic laboratory protocols in epilepsy.

Somatic mosaicism confined to the brain, has been shown to cause focal epileptogenic lesions, and is likely to contribute to the hidden genetics of focal epilepsies (46). Pathogenic variants in the mTOR pathway genes (e.g., *TSC1, TSC2, MTOR, PIK3CA, AKT3*, and *DEPDC5*) are a known cause of malformations of cortical development (MCDs) such as the tuberous sclerosis complex (TSC), focal cortical dysplasia (FCD) and hemimegalencephaly, with associated focal epilepsy (47,48). The two-hit hypothesis applies in some cases, where the MCD is a result of a germline variant and another somatic variant in the brain. The two-hit model may involve variants in the same gene, as reported in patients with *TSC2*-related hemimegalencephaly(49) and DEPDC5-associated FCD (50–54). However, two-hit cases of variants in different genes have also been reported e.g., a TSC patient with a germline variant in *TSC2* and a somatic variant in *DEPDC5* (55) and a patient with hemimegalencephaly with somatic variants in *MTOR* and *RPS6* (56). A recent report from Bennet et al., (2022) described a pathogenic germline mTOR pathway variant (*NPRL3*) and a somatic variant in *WNT2,* both genes in the intersecting WNT (Wingless-related integration site) signalling pathway, detected in a patient with FCD (53). Somatic variants often go undetected even if brain tissue is available for testing, as these may be present at a low allele fraction (e.g., in <5% DNA molecules). Variants in the *SLC35A2* gene encoding a UDP-galactose transporter have been implicated in focal epilepsies with and without detectable brain lesions on MRI or neuropathology (57–59). Recently, brain somatic *SLC35A2* variants have been associated with the clinical phenotypes of EE and drug-resistant focal epilepsy, and mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE) on histopathology(60). It is suggested that as more patients are diagnosed and new genes uncovered, disease-causing somatic variants will gain more prominence as the underlying aetiology of focal epilepsy.

The presence of **modifier variants** has long been hypothesised to contribute towards the genetic and phenotypic heterogeneity of epilepsy, where a pathogenic variant can manifest differently in different individuals even within the same family(61), or variants in different genes result in similar disease. To date, however, there is little reproducible evidence of genetic modifiers in epilepsy. The phenomenon known as intragenic complementation (IGC) occurs when particular combinations of variant alleles at a given locus produce a less-severe phenotype than the same alleles in a homozygous state, or in the presence of non-complementing alleles (62). In a recent report, Hammer and colleagues (63) described a large family with genetic epilepsy with febrile seizures plus (GEFS+), where the loss of function (LoF) p.K1372E *SCN1A* variant segregated with phenotypes ranging in severity from Dravet syndrome to febrile seizures and absence seizures (61). The p.K1372E variant was identified in a heterozygous state in all the affected, as well as two unaffected individuals.

Subsequent ES revealed a second *SCN1A* variant (p.L375S), shared exclusively by the unaffected individuals who also tested heterozygous for the p.K1372E variant, in *trans*. Functional expression analysis showed that co-expression of both variants neutralised the LoF effect of the p.K1372E variant, thereby rescuing the phenotype (63). This discovery presents new evidence towards the possibility that epilepsy phenotypes are influenced by modifier variants, a mechanism previously reported only in animal models (64,65). Demonstrating possible IGC in epilepsy provides a novel framework for pathogenicity in genetic epilepsies, with possible new avenues for treatment.

Exploration of the vast non-coding portion of the human genome is viewed as the next frontier of genetic epilepsy research, now possible with the decreasing costs and increased efficiency of sequencing and data processing. The trends identified in *de novo* variant patterns within the genomic regulatory elements (e.g., promoters and enhancers) in patients with autism and developmental delay, suggest that study of the noncoding genome may go a long way towards better characterisation of complex neurological phenotypes(66,67). In epilepsy, multiple intronic *SCN1A* variants were found to promote inclusion of a **poison exon**, causing DS through nonsense-meditated decay (NMD) and reduced *SCN1A* expression(68). Transcriptome studies have previously identified poison exons in multiple genes, including those involved in neurodevelopment and other ion channel genes (69–71). Future insights into the splicing mechanisms in neuron-expressed genes, may lead to RNA-therapeutic options for epilepsy and associated neurodevelopment disorders. RNA therapeutics to treat splicing disruption are being evaluated in DS (72).

Short tandem repeats (STRs) are scattered throughout the genome, and comprise of repeating sequencing motifs, often used as genetic markers for linkage mapping, human migration studies or human identification testing (genetic fingerprinting) (73). A small proportion STRs have been linked with human disease, where expansion of the repeat number beyond a certain threshold result in disease, mainly involving neurological or neurodevelopmental phenotypes (e.g. Huntington disease, the spinocerebellar ataxias, Fragile X Syndrome and others) (74,75). The disease mechanisms vary but appear to cluster with the motif type and its location in the gene. Intronic expansions usually result in RNA toxicity involving sequestration of transcription factors or aberrant protein folding, which affects the neuronal cells more adversely, owing to their longevity (75). **Intronic repeat expansions** in several genes have been implicated in two epilepsy types: 1) progressive myoclonus epilepsy 1A (EPM1) (Unverricht–Lundborg type), associated with a 12-base repeat motif expansion in the promoter region of the recessive *CSTB* gene (CCCCGCCCCGCG)(76), and 2) familial adult myoclonus epilepsy (FAME), associated with expanded intronic pentamers in five reported loci for FAME1–3 and FAME6–7 (77–79). Both EPM1 and FAME1 display founder effects (80–82). Notably, it has been shown that the FAME1 repeat expansion extends throughout Asia with the same core haplotype surrounding the repeat expansion in *SAMD12*(83,84). These and other pathogenic repeat expansions may be a more common genetic mechanism than previously considered. Importantly, repeat expansions can be a target of gene-based therapies, as shown by the RNA and DNA targeting therapies for Huntington disease, currently in various phases of clinical trials (85,86).

Repeat expansions are not easily detected by current sequencing technologies (87,88). Reliable detection and repeat sizing required for diagnostic testing is still done on locus-by-locus basis, using PCR, repeat-primed PCR

or Southern blotting, which is too expensive and labour-intensive for investigating large cohorts, or disorders with locus heterogeneity (88). In recent years, computational methods have been developed to identify the presence of repeat expansions in standard GS and ES data (89,90). However, the established short-read sequencing platforms (e.g., Illumina) are not capable of reliable detection and characterisation of repeat expansions owing to their large size, low sequence complexity and high GC content (87). Single molecule long-read sequencing (LRS) has proven more suited for this purpose as it generates longer, distinct reads that can be assembled with less ambiguity (91,92). Long-read sequencing is increasingly used in research but the high cost of LRS of whole genomes remains a challenge for the diagnostic setting. Targeted enrichment approaches for genotyping STR loci have been developed, such as the Cas9-based, amplification-free sequencing (93) and more recently, the "ReadUntil" functionality from Oxford Nanopore Technologies (ONT), whereby the sequencing device can be programmed to recognize specific DNA sequence fragments during a sequencing experiment employing standard ONT library preparation (94,95). The extent of the role of repeat expansions in the causation of the more common epilepsies is yet to be determined. However, with the ongoing discovery of novel expansions and epilepsy genes, and the continually improving detection platforms and bioinformatic algorithms, detection of repeat expansion is likely to become routinely incorporated into the analysis protocols for epilepsy.

The majority of epilepsy genes characterised to date, are implicated in the rare monogenic phenotypes, mainly the severe DEEs and some rare familial epilepsies(96,97). However, there are few single gene causes of the more common phenotypes (the generalised and focal epilepsies). The aetiology of these common epilepsies remains largely unsolved, and is likely to be multifactorial and complex in its architecture (98,99). Previous efforts at elucidating the contribution of common variants to epilepsy risk did not yield significant or reproducible results, mainly due to the phenotypic heterogeneity of the cohorts and lack of sufficient power to detect signals of small effect (12,100,101). However, recent international collaborative efforts enabling analyses of large datasets suggest that a significant proportion of genetic risk for generalized epilepsy is explained by common genetic variants (102,103). A GWAS comparing 15212 epilepsy cases (focal, genetic generalized and unclassified epilepsy) with 29677 controls identified 16 statistically significant risk loci (103). The **polygenic risk score (PRS)** analyses to estimate the cumulative risk attributable to common variants (in contrast to the individual contribution of each variant in a GWAS) suggest that common variants may cumulatively explain a quarter of all epilepsy risk, and a third of the risk for generalised epilepsies (27,103). These risk estimates support the hypothesis of a substantial polygenic contribution to the common epilepsies, especially in the generalised epilepsies. An example of possible relevance to future clinical care, is using PRS-based heritability models to predict development of epilepsy after a first unprovoked seizure (27,98). Newly discovered loci and risk variants, may also help to elucidate biological pathways in epilepsy, presenting options for the development of novel treatments.

The role of PRS in the presumed monogenic phenotypes e.g., the DEEs, is increasingly receiving attention. A recent collaborative report investigating data from six cohorts (2,759 cases) of severe phenotypes revealed that the DEEs (and similar forms of epilepsy with ID) have an increased PRS associated with complex epilepsy(104,105). No difference was observed between the polygenic risk burden in the cases with and without an identified deleterious variant(104). This suggests a polygenic contribution to the DEEs and warrants further

investigation. Importantly, polygenic risk scoring requires population-relevant data of sufficient power, again emphasising the need for augmentation of the currently limited data on genetic variation in SSA populations.

The value of defining the **phenotypic spectra** of genetic epilepsies lies in more efficient and accurate diagnosis, treatment and prognosis. A phenotypic spectrum is determined by study of the **natural history** of disease, usually based on studies of case series (though dedicated natural history studies are increasingly being undertaken). A well noted example is *STXBP1*, initially associated with Ohtahara syndrome (106). Subsequent studies showed associations with a range of other DEEs, including West syndrome (107), Lennox-Gastaut syndrome (LGS) (45) and DS(108). *STXBP1* has also been implicated in non-syndromic epilepsies and neurodevelopmental disorders (NDDs) without seizures, such as atypical Rett syndrome (109,110), ataxia-tremor-retardation syndrome without epilepsy(111,112) and ID without epilepsy (113,114). As result, *STXBP1* is now characterised more broadly as a developmental disorders gene, rather than an epilepsy- or a DEE-associated gene (115). Importantly, natural history studies to quantify phenotypic spectra are needed for the design and outcome measures for clinical trials, including endpoints beyond seizure burden, as done for the CDKL5-defficiency disorder (116) and CLN3 Disease (117). Clinical trial readiness and appropriate patient enrolment rely on in-depth understanding of phenotype-genotype correlations and evolution of the condition over time (118).

Whilst technological advancements enabled sequencing of thousands of individuals, resulting in rapid gene and variant discovery, the same level of scalability is difficult to achieve when studying the natural disease course. Natural disease history studies rely on manual noting of signs and symptoms, with problems related to standardisation and subjective assessment. Given the dynamic course and rarity of childhood epilepsies, longitudinal information on the natural history and outcomes is limited. This phenomenon has been termed the "phenotypic bottleneck" in the epilepsies (119), and represents a significant limitation to effective association of genetic aetiologies with clinical information. Critical to overcoming this bottleneck, is the use of standardized phenotyping language for epilepsy outcome measures and accessing the existing large-scale data sources such as the electronic medical records. Novel approaches to integrating genetic diagnoses, natural history, and clinical information include the use of Human Phenotype Ontology (HPO), through data extracted from electronic medical records and large-scale data harmonization(120). The ENIGMA-Epilepsy collaborative is a large quantitative brain imaging consortium established to integrate imaging, genetic, and other clinical data(121). Online repositories for genetic, preclinical, and clinical data for specific neurogenetic disorders are being developed (e.g., http://grin-portal.broadinstitute. org/). Increasingly, advocacy groups are catalysts for natural history studies, aided by larger collaboratives such as the Rare Epilepsy Network (www.rareepilepsynetwork.org) or Rare-X (www.rare-x.org) or in collaboration with industry (e.g., Invitae, https://www.ciiti zen.com/).

2.2.4. Preclinical Disease Models

Faithful recapitulation of disease pathophysiology is crucial for the study of disease mechanisms and subsequent development and pre-clinical testing of new therapies. Development of **transgenic animal models** has facilitated better understanding of the pathogenesis behind NDDs and epilepsy (122). Drosophila and zebrafish transgenic models have been extensively used to study the pathophysiology of neurodevelopment

and epilepsy (123,124), with zebrafish used as a high-throughput screening tool for novel ASMs. However, the significant structural and neurodevelopmental differences between humans and the non-mammalian models renders direct comparison impossible, thus conclusions must be based on careful interpretation of cellular and behavioural readouts (125,126). An improved model for pre-clinical investigation of neurodevelopment and epilepsy, is the transgenic mouse, produced with recent advances in gene editing using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9. Rodent models, somewhat genetically and developmentally similar to humans, are the current model of choice for in vivo testing of gene-based therapies (122,127).

Development of **human *in vitro* models** capable of recapitulating most of the human-specific features of neurodevelopment and epilepsy, should lead to more rapid translation of novel therapeutic approaches into precision therapies. Examples of the developed models include the Chinese hamster ovary or human embryonic kidney cell lines, engineered to express patient genetic material, thus enabling the study of protein function. This has been especially successfully applied to the study of ion channel function as a high-throughput approach, with useful outcomes for precision medicine in the channelopathies (128–130). An important emerging human disease model is the use of induced pluripotent stem cells (**iPSCs**). iPSCs can potentially be differentiated in culture into any human cell type, including all subtypes of neurons, and used to study the effect of genetic epilepsy variants within the unique genomic context of an individual (131,132). Several iPSC-derived neurons have been generated from DS patients (133–135). iPSC-derived cortical excitatory neurons from individuals with *SCN8A*-related epilepsy were used to demonstrate a response to riluzole, with subsequent administration of riluzole to patients with the specific *SCN8A* variants, achieving substantial seizure reduction (136). iPSC-derived neurons have also been used to model other epilepsies, including the DEEs, progressive myoclonic epilepsies, Rett syndrome, TSC and Angelman syndrome(132). An important consideration when using iPSCs-derived models, is that functionally mature neurons are needed to produce patient-relevant cellular phenotypes (133,134). The role of genetic background must also be taken into account, as the disease severity with the same variant is known to differ between disease models and individuals.

Human iPSCs models can also self-organize into three-dimensional (3D) spheroids in culture, forming **cerebral organoids**. Cerebral organoids are an important disease model positioned between the two-dimensional (2D) cell culture and animal models (137,138), used to study a range of neurodevelopmental disorders, including genetic epilepsies. Compared to the iPSC neuronal cultures, organoids can be sustained for longer periods and achieve superior recapitulation of structural features and cellular heterogeneity in the brain They also enable study of neuronal cell types not found in a mouse brain (e.g., outer radial glial cells) (139). An organoid model of TSC allowed for recapitulation of tubers, which are not observed in mouse models (140). The current limitations of cerebral organoids in disease modelling include the inability to generate mature cortical structures with the full range of neuronal and glial cell subtypes, and difficulty in producing consistent phenotypes between experiments. However, these difficulties are likely to be overcome with further development.

2.3 Precision Medicine

Precision Medicine can be described as an approach to prevention and treatment of disease, that incorporates the genetics, environment and lifestyle of an individual, for optimal health outcomes. There is an obvious need for targeted, personalised treatment options in epilepsy, as it is estimated that the response to empirically administered ASMs is suboptimal or fails in approximately a third of the people living with epilepsy worldwide (141).

Classic examples of established targeted treatments can be found among the monogenic phenotypes, particularly the neurometabolic disorders. These include the GLUT1 deficiency syndrome caused by variants in the *SLC2A1* gene, which encodes the glucose transporter protein type 1 (GLUT1), critical for transporting glucose across the blood-brain barrier (Figure 4). A low glucose level in the cerebrospinal fluid is one of the manifestations of the condition. The ketogenic diet (KD) provides an alternative energy source to the neurons, addressing the biochemical defect in the affected individuals, with dramatic seizure cessation or reduction, as well as improvement in cognition and other manifestations (e.g., movement disorders) (142). Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive (AR) neurometabolic disorder, caused by variants in *ALDH7A1,* which encodes antiquitin, a dehydrogenase involved in lysine catabolism and essential for normal neurotransmitter metabolism. PDE is characterized by intractable neonatal seizures that cease upon intravenous administration of pyridoxine (143). Other B6-dependent epilepsies exhibiting a dramatic response to vitamin B6 treatment are PNPO deficiency (144,145) and PLPHP deficiency (146), caused by pathogenic variants in *PNPO* and *PLPBP*, respectively. Figure 4 illustrates established examples if PM in epilepsy, depicted within the ideal PM framework spanning the gene/variant discovery*, in vitro* and *in vivo* models, subsequent drug selection, re-purposing or discovery, culminating in clinical trials or use in patients with variants in the specific gene (Figure 4) (147).

Detailed characterisation of the **functional effects** of genetic variants has enabled targeted use of existing ASMs or repurposing of compounds, particularly well noted among the channelopathies (17,18). Examples include DS (Figure 4), the great majority of which is associated with LoF *SCN1A* variants, encoding the alpha subunit of voltage-gated sodium channel Nav1.1(151). LoF variants result in impaired function of the inhibitory interneurons, hence, therapy with sodium channels blocking agents in *SCN1A*-DS (e.g. carbamazepine) may exacerbate seizures (152). Development of first-line ASMs for DS such as valproic acid, clobazam, fenfluramine, stiripentol, topiramate, and cannabidiol, was based on expert consensus and randomised clinical trials (152,153). Pathogenic variants in *SCN2A,* a type II voltage-gated sodium channel (Nav1.2) gene with a dominant role in neuronal excitability, are associated with a broad range of epilepsy syndromes. This spectrum extends from the benign familial neonatal/infantile epilepsy (BFN/IE) to the severe DEEs, as well as neuropsychiatric disorders (autism, schizophrenia) with and without seizures (154). Typically, *de novo* gain of function (GoF) *SCN2A* variants tend to cluster with the neonatal/infantile-onset phenotypes (<3 months) such as the benign familial infantile seizures and DEE and respond well to sodium channel blocking agents (SCBs), such as oxcarbazepine. In contrast, *SCN2A* LoF variants are more frequently associated with later-onset epilepsy (>3 months) in patients with DD, ASD and/or epileptic encephalopathies, in whom SCBs should be avoided (128).

Similar considerations regarding the use of SCBs apply to the *de novo SCN3A* and *SCN8A* epilepsies, also characterized by a wide clinical spectrum ranging from DD without seizures to severe DEE (155,156).

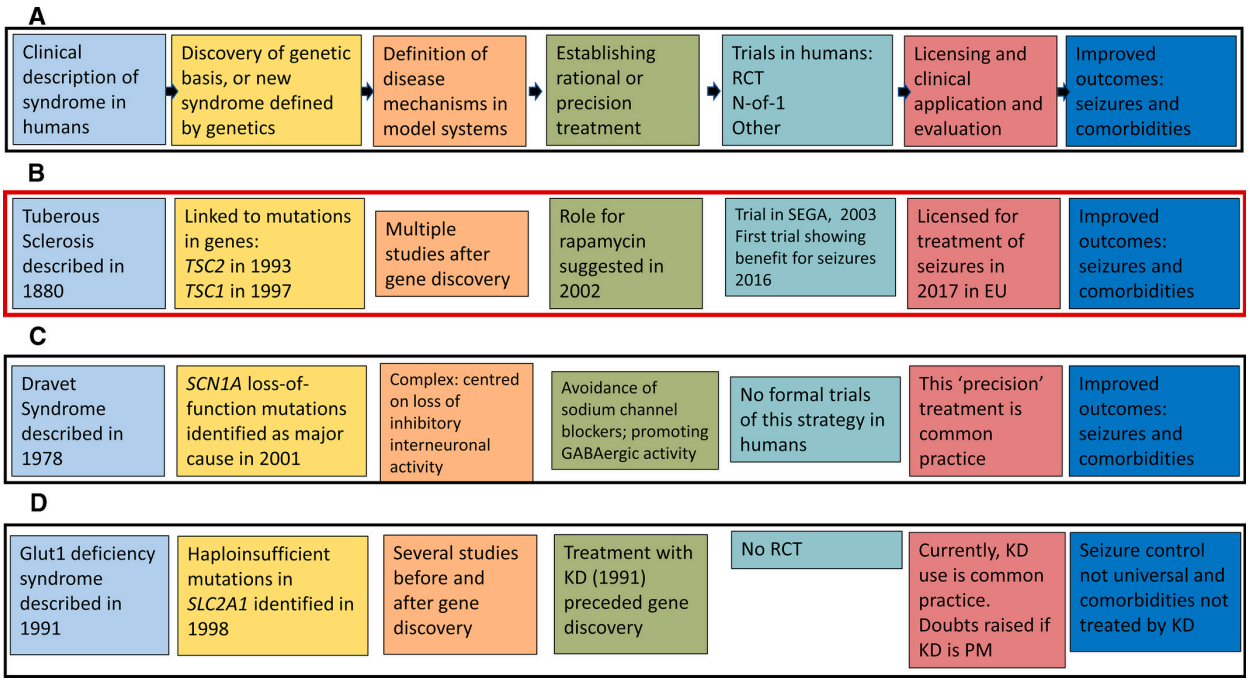

Figure 4. **Typical examples of PM in epilepsy.** A: the "ideal" PM paradigm with a linear progression from clinical description of an epilepsy, determination of its genetic cause, definition of disease mechanisms, establishing the basis of a rational treatment, subsequent clinical trials, licensing, and seizure-free outcomes with improvements of comorbidities. B: The PM paradigm in tuberous sclerosis complex (TSC). C: The PM paradigm in *SCN1A*-DS. The strategy of avoiding sodium channel–blocking ASMs is the typical practice, with published evidence of benefit (though not from formal trials) (148,149). D: The PM paradigm in GLUT1 deficiency syndrome. Before discovery of *SLC2A1,* individuals with the clinical syndrome were treated with KD on the basis of biochemical testing. KD is the standard treatment for GLUT1 deficiency disorder, although there have been no randomized controlled trials. Its position as a PM has been debated (150). EU: European Union; KD: ketogenic diet; PM: precision medicine; RCT: randomized-controlled trial; SEGA: subependymal giant cell astrocytoma (adapted from Sisodiya S.M., 2020 (147); permission through RightsLink).

Understanding the functional effect of the putative variant is therefore an important factor in the choice of therapy. However, electro-physiological studies are time and resource-intensive, and generally only performed for selected variants in a research setting. This happens outside of the clinically relevant time-frames, which is problematic, especially for the severe early-onset phenotypes where the correct treatment from the onset is critical. Brunklaus and colleagues designed a free *in silico* tool, for predicting the functional effect of variants across the sodium channelopathies, based on the evolutionarily conserved nature and biophysical similarities of the voltage-gated sodium channels (157). The authors acknowledge that variant categorisation as LoF, GoF or mixed is simplistic and does not always fully reflect the biophysical complexities of the voltage gated sodium channels. Nonetheless, together with the proposed key indicators, the tool offers a pragmatic approach to functional variant categorisation in the absence of gold-standard functional data (http://SCN-viewer.broadinstitute.org). Similar approaches aimed at non-ion channel genes are under development, notably, the Epilepsy Multiplatform Variant Prediction (EpiMVP) Centre Without Walls (https://epimvp.med.umich.edu/). EpiMVP is a multicentre collaboration which uses cell culture models, whole-animal models, genome editing techniques and machine learning algorithms for the development of a reliable variant-effect prediction tool in genetic epilepsies.

These big-data, machine-learning approaches designed to assess response to treatment are challenged by the wide phenotypic spectra and certain aspects of the natural disease histories. For example, some patients with the early-onset *SCN2A*-related epilepsies may become seizure-free regardless of treatment, whilst the developmental and movement disorders persist. Moreover, the complexities of ion channel dysfunction and the effects exerted by different genetic variants have not been fully explained (119). Therefore, the current therapeutic approaches for the channelopathies, whilst targeted, do not quite fit the description of PM, as their development was not based on fully elucidated disease mechanisms, and administration remains largely empirical and often suboptimal (158).

Epilepsy gene discovery has prompted **repurposing of drugs** not originally intended to treat seizures. Among successes is the already mentioned fenfluramine, a serotonin releasing agent previously Food and Drug Administration (FDA)-approved for use as an appetite suppressant in the treatment of obesity. It was subsequently withdrawn due to cardiovascular toxicity before being repurposed for other indications (159). Fenfluramine was approved for treatment of Dravet syndrome by the United States FDA and the European Commission in 2020, following clinical trials demonstrating reduced convulsive seizures and supportive safety data (160). An example of limited success is the treatment of *KCNT1*-realted epilepsy with quinidine, an established antiarrhythmic drug. Pathogenic *de novo KCNT1* variants are most commonly associated with epilepsy of infancy with migrating focal seizures (EIMFS) (161), though the gene is also implicated in other, severe epilepsy syndromes (162). The functional effect of the disease-causing *KCNT1* variants was shown to be a GoF, resulting in a constitutive activation of the SLACK channel, potentially amenable to treatment with quinidine (163). Case reports of quinidine treatment in *KCNT1*-related epilepsy, described a dramatic reduction in seizure burden. This initial success, however, could not be reliably reproduced in clinical trials, because of heterogeneity in blood-brain barrier penetration, quinidine cardiotoxicity and variable responsiveness in different patients with different variants, and no significant reduction in seizures overall (164,165). Although quinidine treatment for *KCNT1*-related epilepsy has not been entirely dismissed, any future use will require careful patient selection, based not only on the identified pathogenic variant but also on specific electroclinical characteristics, which are not yet fully understood. This experience is seen as a cautionary tale in repurposing drugs for use as PM in epilepsy, emphasising the importance of carefully and diligently conducted clinical trials, even for repurposed therapies (147). It is emphasised therefore, that human **clinical trials** for drug repurposing in epilepsy should maximize sample size and adhere to standardized protocols. Functional characterization of the putative variants (GOF or LOF), as well as exquisite phenotyping are mandatory. The small samples sizes may warrant alternative clinical trials designs, for increased rigor and generalizability (27,147,166).

The PM paradigm in epilepsy is challenged by its marked genetic and phenotypic heterogeneity, and incomplete understanding of the disease mechanisms, even among the monogenic phenotypes. Despite the dynamic progress in gene discovery and growing understanding of disease causation, PM in epilepsy is still only possible for a small subset of patients, mainly those with monogenic DEEs (17). Treatment for most people living with epilepsy, especially the most common phenotypes, remains imprecise (158). The reasons behind the failures (and successes) of PM in some cases are not fully understood but are likely to result from as yet unexplained factors acting in addition to the identified pathogenic variant in the individual. Owing to this limited understanding,

many of the current targeted treatments fall somewhat short of the true definition of PM. It has been suggested that a rigorous framework is required to determine a PM approach. This should take into account the complete context of the putative genetic variant, its functional effects, as well as the effects of the genomic and other measurable environments affecting the individual. S.M. Sisodiya (147) proposed that the following criteria should be met to judge if a therapeutic approach qualifies as PM:

"1. There is a robust understanding of the necessary and sufficient mechanisms leading from putative cause to clinically manifest disease.
2. *The postulated disease mechanisms should be measurable at the level of the necessary and sufficient elements in the disease pathophysiology.*
3. *The precision treatment strategy should be justifiably based on the understanding of underlying mechanisms.*
4. *The strategy should improve clinical outcomes, with parallel evidence at the required mechanistic level that the putative pathophysiology has been corrected or addressed.*
5. *Failure of a precision therapy should be explained on the basis of the postulated disease mechanisms."*

[Copied from: Sisodiya SM. Precision medicine and therapies of the future. Epilepsia [Internet]. 2021 Mar 1 [cited 2022 Mar 13];62(S2):S90–105. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/epi.16539]

The author argues that inasmuch as the above criteria may appear overly-demanding, this level of evidenced understanding is required to prove that the treatment has the hypothesised action and effect, and that it is indeed precise and can be reliably prescribed as such (147).

Another approach to precision treatment is **gene-based therapy**, which has shown promise in genetic disease, including neuromuscular and NDDs e.g., Duchenne muscular dystrophy (DMD) (167,168), spinal muscular dystrophy (SMA) (169), Angelman syndrome (170). The premise of gene-based therapy is to treat genetic disease by replacing or repairing the faulty gene, thereby restoring expression and physiological function. The goal is to achieve durable expression at a level sufficient to ameliorate or cure disease symptoms with minimal adverse events. Gene replacement, as the traditional gene therapy strategy, entails delivery of the supplemental transgene to the defective cells. This approach has seen some success, with approval from the European Medicines Agency (EMA) and the U.S. FDA) for several genetic disorders, including SMA (171). However, gene therapy for epilepsy has yet to translate into clinical application. A major challenge is the so-called packaging limit of the current gene delivery methods mostly employing adeno-associated virus (AAV) vectors, which excludes delivery of larger genes, such as the ion channels. Alternative approaches are therefore required for these genes, such as the ETX101 therapy soon to be tested in clinical trials for DS (https://clinicaltrials.gov/ct2/show/NCT05419492). ETX101 is designed to deliver a transgene encoding an engineered *SCN1A*-specific transcription factor (eTF^{SCN1A}) to upregulate expression of the endogenous *SCN1A* gene. Expression of the transgene is controlled by a GABAergic inhibitory neuron-selective regulatory element (RE^{GABA}). This showed promising results in the DS mouse model (172). Expanded use of AAV vectors requires overcoming the vector packaging limit (or reducing the package size) and ensuring safe systemic delivery.

A large proportion of pathogenic variants in the ion channel genes result in toxic GoF, where supplementation is not appropriate, but rather, necessitates tools aimed at selective reduction in gene expression. Antisense oligonucleotides (ASO) therapy employs synthetic, short single-stranded oligodeoxynucleotides to target specific mRNA transcripts and restore or modify protein expression through mechanisms such as modified mRNA splicing or mRNA degradation (173). Reduced premature death and seizure burden were observed in knock-in mouse models with human *SCN2A* or *SCN8A* GoF variants, upon treatment with ASOs designed to lower expression (174,175). ASOs can also be used to increase gene expression, as demonstrated by Han *et al*.(2020), with ASOs designed to enhance *SCN1A* gene expression by modulating non-productive splicing events, achieving augmentation of the gene output, with reduced seizures and mortality in a DS mouse model (72). Clinical trials are currently underway in the United States and United Kingdom (https://clinicaltrials.gov/ct2/show/NCT04442295). Also promising is the direct gene repair or editing using CRISPR-Cas9, though significant safety aspects such as off-target effects and potential adaptive immunity to forms of Cas9 owing to the need for frequent administration, must still be addressed before testing in human trials. A dead Cas9 (dCas9)-mediated promoter-enhancing strategy to enhance *SCN1A* expression was effective in vitro and in a DS mouse (176). AAV-based approaches may not be limited to gene replacement, and include the use of AAV vectors for ASOs and CRISPR-Cas9 systems. This has not yet been tested in humans for genetic epilepsy, but has been tested in preclinical models to correct overexpression of a potassium channel (177) and replenishment of the endogenous antiseizure neuropeptide preprodynorphin (178).

Safety and physical accessibility are the major obstacles in gene-based therapy for epilepsy, as the central nervous system (CNS) is the treatment target and delivery must efficiently and specifically access the whole brain. Currently, most gene-based approaches require intrathecal administration due to poor CNS penetration and stability. Furthermore, phenotype rescue or amelioration is likely to require administration within a critical developmental time-frame (179), and may not reverse all deleterious phenotypes of a pathogenic variant, particularly those arising during early neurodevelopment. Altering the expression levels of genes involved in the neurodevelopment will also require careful dosing to avoid introducing new pathologies (180). Therefore, further work is required to render drug-based therapies safe and effective for clinical use. However, the ability to adjust the expression of specific gene targets presents a therapeutic option for cases considered untreatable with pharmacotherapy, and ineligible for surgery. Similar to all precision treatment strategies in epilepsy, identification of the underlying genetic cause is an essential part of the gene-based therapy approach.

Identifying the genetic cause of epilepsy in an individual remains the first step in assessing options for precision treatment. Enabling and optimising this first step for DEE patients in Africa and other resource-constrained settings, was the main focus of this research study.

3. PILOT STUDY

The main study was piloted with a project involving 22 SA children clinically diagnosed with DS. The reasons behind choosing this phenotypically and genetically well-defined patient sub-group were: 1) validating performance of the locally accessible Ion Torrent NGS platform by testing a group of individuals who were likely to harbour pathogenic variants in one gene (i.e., SC*N1A*) and whose results could then be compared to those subsequently obtained with the Illumina platform in the main study, and 2) determining if *SCN1A* variants were indeed the primary cause of DS in SA patients and if the phenotypic determinants were similar to those described in international cohorts. This was successfully achieved on both counts and the findings were published in November 2018 in *Seizure - European Journal of Epilepsy*.

Journal: *European Journal of Epilepsy – Seizure.*

Title: Dravet Syndrome in South African Infants: Tools for an Early Diagnosis.

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ABSTRACT

Purpose:

Dravet syndrome (DS) is a well-described, severe genetic epileptic encephalopathy with an increased risk of sudden unexpected death in epilepsy (SUDEP). The incidence and genetic architecture of DS in African patients is virtually unknown, largely due to lack of awareness and unavailability of genetic testing. The clinical benefits of the available precision medicine approaches to treatment emphasise the importance of an early, correct diagnosis. We investigated the genetic causes and clinical features of DS in South African children to develop protocols for early, cost-effective diagnosis in the local setting.

Method

We selected 22 South African children provisionally diagnosed with clinical DS for targeted resequencing of DS-associated genes. We sought to identify the clinical features most strongly associated with *SCN1A*-related DS, using the DS risk score and clinical co-variates under various statistical models.

Results

Disease-causing variants were identified in 10 of the 22 children: nine *SCN1A* and one *PCDH19*. Moreover, we showed that seizure onset before 6 months of age and a clinical DS risk score of >6 were highly predictive of *SCN1A*-associated DS. Clinical reassessment resulted in a revised diagnosis in 10 of the 12 variant-negative children.

ABSTRACT cont.

Conclusion

This first genetic study of DS in Africa confirms that *de novo SCN1A* variants underlie disease in the majority of South African patients. Affirming the predictive value of seizure onset before 6 months of age and a clinical DS risk score of >6 has significant practical implications for the resource-limited setting, presenting simple diagnostic criteria which can facilitate early correct treatment, specialist consultation and genetic testing.

Highlights

- DS in Africa is underdiagnosed due to lack of awareness and access to testing.
- *SCN1A* variants underlie DS disease in the majority of South African patients.
- Seizures before 6 months of age and a clinical risk score of >6 suggest *SCN1A*-DS.
- These simple diagnostic tools can guide treatment and prompt genetic testing.

1. INTRODUCTION

Dravet syndrome (DS) (OMIM 607208), previously described as severe myoclonic epilepsy of infancy (SMEI) is a severe genetic epilepsy with associated encephalopathy [1]. Early clinical presentation of DS is characterised by the onset of prolonged, febrile and afebrile generalized clonic or hemiclonic seizures in an otherwise normally developing infant. Seizures are usually resistant to typically prescribed antiepileptic drugs (AEDs) and evolve with the disease progression to include myoclonic, atypical absences and focal seizures. After this initial phase, the clinical presentation becomes less distinctive and the opportunity to recognize the condition early may be missed. Life-threatening episodes of status epilepticus (SE), seizure-related accidents and sudden unexpected death in epilepsy (SUDEP), all contribute towards a significantly increased premature mortality among individuals with DS [2,3]. An important reason for early recognition of DS, is the contraindication of treatment with sodium channel inhibitors (e.g., carbamazepine, oxcarbazepine, lamotrigine), as this may worsen the condition [4,5]. Other contraindications include chronic use of benzodiazepine (BZ), which may facilitate encephalopathy and resistance to CBZ administered for status epilepticus (SE) [6].

DS progresses in three stages: the first diagnostic “febrile stage” is marked by frequent, prolonged febrile seizures in the first year of life; the second “worsening stage” occurs between the ages of 1 and 5 years with frequent seizures and episodes of status epilepticus, behavioural deterioration and neurological signs, followed by the third “stabilization stage” characterized by a decrease in convulsive seizures which occur mainly during sleep. During this last stage, seizures continue to impact on the child’s quality of life, though myoclonic and absence seizures may disappear. Neurological development may improve but a variable degree of cognitive impairment persists, often with challenging behavioural issues. Ataxia and gait problems become a major concern [1]. At present, the realistic objective of treatment is cessation of prolonged seizures, reduced seizure frequency and cognitive and motor sequalae [6]. The degree of success, however, is wholly dependent on early correct diagnosis and appropriate intervention.

Over 80% of DS cases are associated with *de novo* variants in the *SCN1A* gene (OMIM 182389), which encodes the alpha subunit of the sodium ion channel [1]. The majority of the remaining DS patients do not

carry currently identifiable variants, though some children harbour pathogenic variants in other ion and non-ion channel genes [7]. This relative genetic homogeneity holds DS apart from most other developmental and epileptic encephalopathies (DEEs), which are highly genetically heterogeneous [8,9]. Careful clinical correlation is important, as *SCN1A* variants are also found in other severe epilepsies [e.g., epilepsy of infancy with migrating focal seizures (EIMFS)] [10], as well as less severe epilepsy phenotypes such as familial febrile seizures (FFS) [11] or genetic epilepsy with febrile seizures plus (GEFS+) [12]. The factors predicting long-term developmental outcome remain unclear but an early diagnosis and seizure control may delay or prevent the onset of DEE and mitigate the outcomes [5,13].

The incidence of DS in high-income countries (HICs) is estimated to range between 1 in 15 700 and 1 in 40 900 live births [1]. At present, the incidence of DS in Africa is unknown, due to virtual absence of genetic testing or epilepsy research. However, given that most *SCN1A* variants arise *de novo*, we expect the incidence of DS in Africa to reflect that of international studies. Whilst Africa, particularly sub-Saharan Africa (SSA), bears the highest burden of epilepsy in the world [14], genetic epilepsy is among the most underdiagnosed and under-investigated disorders on the continent. In a setting where seizures are frequently a result of endemic parasitic disease, central nervous system (CNS) infections, traumatic brain injury or perinatal insults, a diagnosis of a genetic epilepsy is rarely considered. Acute symptomatic seizures and febrile seizures are frequently assumed to be due to malaria, limiting the search for other causes [15]. Lack of awareness, limited specialist expertise, suboptimal health infrastructure and unavailability of diagnostic testing all contribute towards this void in knowledge and medical care. The extensive evidence of the genetic contribution to many epilepsy phenotypes, and the clinical utility of testing, especially relevant to early-life epilepsies, has informed the diagnostic laboratory protocols of many HICs. [16–18]. It is important to ensure that African patients also benefit from this knowledge and that new knowledge is gained through research on the highly genetically diverse populations of Africa [19]. In this study, we collected clinical and electroclinical information and performed genetic testing on a cohort of 22 South African infants diagnosed with Dravet or Dravet-like syndromes. Our main aim was characterization of the genetic landscape of DS in South Africa (SA) for the purpose of drawing up clinical and molecular diagnostic protocols for early, cost-effective diagnosis of DS in the local setting, including retrospective cascade counselling and patient follow-up. To our knowledge, this is the first report correlating phenotypic and genetic aspects of DS in Africa.

2. METHODOLOGY

2.1. *Cohort recruitment*

Infants with provisional clinical diagnoses of DS were recruited over a period of six months by clinicians affiliated to or working in the Paediatric Neurology service at the Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town. The patients were referred to the Epilepsy Clinic at the RCCWM, which is the only specialist paediatric epilepsy clinic in sub-Saharan Africa. The cohort comprised of 22 unrelated infants (12 males and 10 females) of European (n=5); Indigenous Black African (n=10); and Mixed (n=7) ancestries, as per parental self-classification. Inclusion was based on a history of infantile onset (before two years of age), recurrent complex febrile seizures (FS) with prior normal development [20]. Structural or metabolic causes were previously excluded. The inclusion criteria were purposefully broad and simple to

enhance recruitment. Peripheral blood was drawn from the infants and parents, after obtaining parental written consent. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 232/2015).

2.2. *Genetic analysis*

Genomic DNA was isolated from peripheral blood (2–5ml) of the probands and parents (where available). DNA isolation and the integrity checks were performed using standard methods (NanoDrop™1000 and, Qubit® dsDNA HS (High Sensitivity) Assay, ThermoFisher Scientific, USA). The cohort was initially tested locally (Division of Human Genetics, University of Cape Town (UCT)), where the Ion Torrent™ PGM platform (ThermoFisher Scientific) was used to re-sequence six genes previously reported to carry pathogenic variants in children with DS (*SCN1A, GABRA1, GABRG2, STXBP1, HCN1 and PCDH19*). The AmpliSeq™ Designer Software v4.47 (ThermoFisher Scientific, USA) was used to design two pools of primers for a total of 161 amplicons, predicted to capture 99,08% of all the coding exons (with 100% capture for the *SCN1A* gene specifically), each flanked by ten bases of intronic sequence (RefSeq. hg19 build), to be sequenced at a minimum 100x depth of coverage. The NGS library was prepared on the Chef DL8 using the Ion AmpliSeq™ Kit (ThermoFisher Scientific, USA) according to the manufacturer's protocol. Basic NGS quality assessment, read alignment, variant identification, annotation, prioritisation, and filtering was performed by the Ion Reporter™ cloud-based software (ThermoFisher Scientific, USA). The VCF files were then used for further manual variant filtering and prioritisation. Only nonsynonymous, splice-site and frameshift variants not found in the ExAC v0.3, ESP6500 or 1000 Genomes databases were assessed further [21–23]. All putative variants were confirmed by Sanger sequencing. Segregation analysis was done in all cases where parental samples were available. All variant-negative samples were tested with the multiple ligase-dependent probe amplification (MLPA) assay for exonic deletions/duplications in the *SCN1A* gene (P137-B2 probe mix, MRC-Holland).

To validate our findings on the Ion Torrent™PGM system, the DS cohort was re-tested on the Illumina HiSeq™ platform at the University of Washington (Seattle, USA), as part of a larger project investigating the genetic causes of DEEs in South African patients (ongoing). The single molecule Molecular Inversion Probe (smMIP) technology was employed as previously described [24] to capture all exons and intron-exon boundaries (5-bp flanking sequences) of the target genes at capture, including *SCN1A, GABRA1, GABRG2, STXBP1, HCN1 and PCDH19* (RefSeq, hg19 build) [25]. Sequencing was performed at 98% capture and 40X minimum depth of coverage. NGS quality assessment, read alignment, depth of coverage, variant identification, annotation, prioritisation, and filtering was also performed using previously published methods [25–27]. The VCF files were then subject to further manual variant filtering and prioritisation.

2.3. *Pathogenicity assessment of variants*

Only nonsynonymous, splice-site and frameshift changes were considered for pathogenicity assessments (Table 2). Variants were classified according to the interpretation guidelines from the American College of Medical Genetics and Genomics–Association for Molecular Pathology (ACMG–AMP) [28]. Briefly, a variant was classified as likely/pathogenic if it arose *de novo* (or from a somatic mosaic parent) and was not found in the publicly available control datasets (ExAC v0.31, ESP6500, 5000 Genomes, gnomAD r2.0.2) [21–23].

In cases where DNA from both parents was unavailable for segregation analysis, likely/pathogenicity was inferred on the basis of (1) the variant type (truncations and splice variants were seen as likely pathogenic), (2) recurrence (previously recorded as disease-causing in the literature or disease databases (3) analysis with in silico pathogenicity prediction tools (CADD, PolyPhen-2, and GERP), where all outputs had to be in agreement (CADD > 25, PolyPhen-2 > 0.9, and GERP > 5). Microsatellite analysis (Authentifiler™ PCR Amplification kit, ThermoFisher Scientific) was performed on of all parents of probands with a *de novo* variants to confirm parentage.

2.4. *Clinical data assessment*

Clinical demographics, seizure semiology, seizure evolution and treatment history were collected both prospectively and retrospectively by clinical assessment, parent/guardian interview and review of patient records (Table 3). A clinical risk score for progression to DS after an initial complex febrile seizure described by Hattori et al., was determined for each patient [29]. The score takes into account the age at seizure onset, total number of seizures before one year of age, total number of prolonged seizures (longer than 10min), and the seizure type and trigger (Table 1). The clinical score was then compared to the clinician's level of confidence in the diagnosis of DS: definitely compatible with DS or possible DS. It was also correlated with the presence/ absence of an *SCN1A* variant.

2.5. *Statistical analysis*

Statistical comparison of the clinical demographics, seizure semiology, seizure evolution and treatment history was made between the group of patients with *SCN1A* variants and the group with no identified variants (Table 3), using R [30]. This was intended to highlight any possible statistically significant associations between specific clinical features, and the presence/ absence of an *SCN1A* variant. Fisher's exact test was used where there were two nominal variables. To permit nonparametric analysis of the two groups without assuming normal distribution of values the Mann-Whitney U test was used for other parameters.

Table 1. **Predictive risk scoring for an early diagnosis of DS**, proposed by Hattori et al. [29]. A total cumulative score of ≥6 strongly increases the risk of DS [29].

Predictive risk factors	**Risk score**
Age of febrile seizure onset <7 months	2
A total number of seizures >5	3
Prolonged seizures lasting >10 min	3
Hemiconvulsions	3
Focal-onset seizures	1
Myoclonic seizures	1
Hot water–induced seizures	2

3. RESULTS

3.1. *Genetic analysis*

Pathogenic changes were found in 10 out of 22 patients: nine carried *SCN1A* variants (four missense, three frameshift and two nonsense) and one female carried a heterozygous nonsense variant in the *PCDH19* gene. The specific coverage achieved for the coding region of the *SCN1A* gene (26 exons) was 100% capture and a>100X depth of coverage on Ion Torrent and>40X unique capture with smMIPs. *De novo* variants could be shown in only five patients, as DNA from both parents was not available in the remaining cases (Table 2).

3.2. *Statistical analysis*

The key findings in the two main groups, namely, the *SCN1A*-positive group (n=9) and the variant-negative group (n=12), are summarised in Table 3. Median age at the time of the last clinic review was 24 months (range 19–51.75) for variant-negative patients and 75 months (range 25–103) for the *SCN1A* variant-positive patients. The analysis revealed a number of significant differences, the most notable of which were the DS clinical score and the age at seizure onset (AAO). The high DS clinical risk score among the *SCN1A*-positive group (median score 9.00, range 8.00–11.00) was consistent with the level of confidence in the diagnosis of DS among the *SCN1A*-positive patients (definitely DS in 6/9 (68%)), compared to the variant-negative patients [definitely DS in 2/12 (17%)]. Age at first seizure was shown to be markedly younger in the *SCN1A*-positive group, with a median of four months (range 3–6) months, compared to 12 months (range 8.75–13.25) in the variant-negative group. The *SCN1A*-positive group were also more likely to have suffered prolonged febrile seizures (>10min) or febrile SE. Despite a range of seizure types described in the study cohort, significance was only found for myoclonic and focal seizures in the *SCN1A*-positve group.

Regarding interventions, the *SCN1A*-postitive group was more likely to receive a combination of AEDs (eight out of nine *SCN1A*-positive patients), whilst 11 out of 12 variant-negative children were managed effectively with monotherapy. No significant differences between the two groups were noted in the median number of AEDs trialled or the degree of seizure control achieved. Whilst there was no difference in the developmental function before seizure onset, developmental delay was more likely in the *SCN1A*-positive group after seizures onset. Similar findings were noted for subsequent speech, behaviour and features of the Autism Spectrum Disorder (ASD), based on neurodevelopmental assessments. The *SCN1A*-positive children were significantly more likely than the variant-negative group to require ancillary support and to be placed in special-needs schools. They were also better attendees to the Neurology service, with more frequent hospital visits related to the challenges of managing intractable seizures and the associated complications.

Long term follow-up enabled clinical reassessment of the variant-negative group with a revised diagnosis in ten patients: seven were re-diagnosed with febrile seizures plus (FS+) and one with early onset epileptic encephalopathy (EOEE). Perinatal insult and Moyamoya disease were determined as the cause of seizures in the remaining two cases. It was also noted that out of 11 Indigenous Black African children in our study (45% of the cohort), only one carried a *SCN1A* variant [LRG_8t1(*SCN1A*):c.5314G>A, p.(Ala1772Thr], with the other *SCN1A* variants detected in children of European (four) and Mixed Ancestry (four).

Table 2. **Pathogenic variants identified by targeted NGS analysis of known Dravet syndrome genes.**

#	Sex	GENE	cDNA level[a]	Protein level	Variant type	Detection method	Novel / known	Variant classification [28]	SIFT/ PolyPhen	GERP	Segregation	Score [29]	AAO (mths)	EXaC MAF [22]
1	F	*SCN1A*	c.5314G>A	Ala1772Thr	missense	Ion Torrent PGM and smMIPS Illumina	rs121917980	Pathogenic	0/1	5,69	no parental DNA	10	2	none
2	F	*SCN1A*	c.3007delA	Ile1003fs	frameshift	Ion Torrent PGM and Sanger Sequencing[b]	Novel	Likely Pathogenic	–	–	no parental DNA	7	4	none
3	M	*SCN1A*	c.664C>T	Arg222Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	rs121918624	Pathogenic	0/1	5,77	*de novo*	15	5	none
4	M	*SCN1A*	del exons 5–8 (c.(602+1_603-1)_(1170+1_1171-1)del)	Tyr202Hisfs*10	frameshift	smMIPS Illumina and MLPA[c]	Novel	Pathogenic	–	4,73	*de novo*	7	8	none
5	F	*SCN1A*	c.2552G>C	Arg851Pro	missense	Ion Torrent PGM and smMIPS Illumina	Novel [d]	Likely Pathogenic	0/0.999	4,75	*de novo*	11	3	none
6	F	*SCN1A*	c.4016T>G	Val1339Gly	missense	Ion Torrent PGM and smMIPS Illumina	(HGMD, Meng et al., 2015)	Pathogenic	0/0.993	4,25	*de novo*	9	6	none
7	F	*SCN1A*	c.5236G>T	Gly1746Trp	missense	Ion Torrent PGM and smMIPS Illumina	Novel	Likely Pathogenic	0/1	5,69	no parental DNA	12	6	none
8	F	*SCN1A*	c.4352_4357 delACTTTG	Tyr1451fs	frameshift	Ion Torrent PGM and smMIPS Illumina	Novel	Likely Pathogenic	–	5,3	no parental DNA	8	4	none
9	M	*SCN1A*	c.1129C>T	Arg377Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	rs794726799	Pathogenic	–	3,34	no parental DNA	9	2	none
10	F	*PCDH19*	c.2371C>T	Gln791Ter	nonsense	Ion Torrent PGM and smMIPS Illumina	Novel	Likely Pathogenic	–	5,64	*de novo*	9	7	none

[a] *SCN1A* RefSeq NM_006920.4, LRG_8t1; *PCDH19* RefSeq NM_020766.2; [b] failed on smMIPS; [c] confirmed with MLPA; [d] previously reported pathogenic variant in this position: p.Arg851Gln (rs121918785); AAO: age at seizure onset; smMIPS: single molecule Molecular Inversion Probe.

The diagnosis of DS was subsequently revised for eight of the nine variant-negative black patients (six FS+, one perinatal insult and one Moyamoya disease). Also, closer scrutiny of the clinical demographics showed that the median clinical score among the variant-negative indigenous black African children was six (range 0–8), and the median age of onset was 12 months (range 3–17 months) (not included in Table 3).

Table 3. **Statistical comparison of the clinical demographics between the variant-negative and the *SCN1A* variant-positive patient groups**.

Clinical and demographic features	**No variant (N = 12)**	***SCN1A* variant (N = 9)**	**p-value**	**test**	**Odds ratio (95% CI)**
DS Risk Score (median, IQR)	**5.50 (3.75, 6.00)**	**9.00 (8.00, 11.00)**	**0,001**	Mann-Whitney-Wilcoxon	
Certainty of DS diagnosis					
Possibly	10	3	0,032	Fisher exact	0.12 (0.01,1.06)
Definitely	**2**	**6**	**0,032**	Fisher exact	**8.73(0.94,135.76)**
Sex	5F:7M	4F:5M	1,000	Fisher exact	1.11(0.14,8.46)
Age at onset (median, IQR in mths)	**12.00 (8.75, 13.25)**	**4.00 (3.00, 6.00)**	**0,003**	Mann-Whitney-Wilcoxon	
Age last seen (median, IQR in mths)	**24.50 (19.00, 51.75)**	**75.00 (25.00, 103.00)**	**0,036**	Mann-Whitney-Wilcoxon	
Seizure History					
No of prolonged (>10 min)	**0.00 (0.00, 1.25)**	**4.00 (2.00, 5.00)**	**0,012**	Mann-Whitney-Wilcoxon	
Status epilepticus	**0.00 (0.00, 1.25)**	**2.00 (1.00, 5.00)**	**0,058**	Mann-Whitney-Wilcoxon	
Seizure type					
Hemiclonic	0	2	0,171	Fisher exact	-
Focal	**3**	**6**	**0,087**	**Fisher exact**	**5.44(0.66,60.59)**
Myoclonic	**3**	**7**	**0,030**	**Fisher exact**	**9.14(1.01,140.65)**
Atypical abs	3	0	0,229	Fisher exact	-
Typical abs	0	3	0,063	Fisher exact	-
Tonic	2	2	1,000	Fisher exact	1.40(0.08,23.88)
GTCS	10	7	1,000	Fisher exact	0.71(0.04,12.07)
Atonic	2	3	0,611	Fisher exact	2.39(0.21,36.66)
Seizure triggers					
Fever	12	9	1,000	Fisher exact	
Hot water	**0**	**3**	**0,063**	**Fisher exact**	**-**
Light	0	3	0,063	Fisher exact	-
Family history	5	3	1,000	Fisher exact	0.71(0.08,5.73)
Peri or postnatal complications	2	1	1,000	Fisher exact	0.64(0.01,14.44)
Investigations (abnormal)					
Metabolic	0	0	1,000	Fisher exact	-
Neuroimaging	4	2	0,659	Fisher exact	0.59(0.04,5.70)
EEG	6	5	1,000	Fisher exact	1.24(0.16,9.89)
Antiepileptic drugs					
Monotherapy	11	1	0,0004	Fisher exact	0.02(0.0003,0.28)
2+ agents	**1**	**8**	**0,0004**	**Fisher exact**	**55.66(3.54,4052.99)**
KD	0	3	0,063	Fisher exact	-
Surgery	0	0	1,000	Fisher exact	-
Seizure control					
Controlled	4	1	0,338	Fisher exact	0.27(0.005,3.53)
Partial control	8	5	0,673	Fisher exact	0.64(0.8,5.23)
Poor control	0	3	0,063	Fisher exact	0.27(0.01,3.53)
Maximum trialled AEDs	2.00 (1.00, 2.25)	3.00 (2.00, 3.00)	0,083	Mann-Whitney-Wilcoxon	
Development (n = abnormal)					
Before seizure onset	1	1	1,000	Fisher exact	1.35(0.02,117.49)
Post seizure onset	**4**	**9**	**0,005**	**Fisher exact**	**-**

Table 3. cont.

Clinical and demographic features	No variant (N = 12)	*SCN1A* variant (N = 9)	p-value	test	Odds ratio (95% CI)
Specific concerns					
Gait	**2**	**5**	**0,159**	**Fisher exact**	**5.66(0.61,83.65)**
Speech	**3**	**7**	**0,030**	**Fisher exact**	**9.14(1.012,140.65)**
Sleep	0	3	0,063	Fisher exact	_
Behaviour	**3**	**7**	**0,030**	Fisher exact	**9.14(1.01,140.65)**
ASD	0	1	0,429	Fisher exact	-
Interventions					
Physiotherapy	**3**	**6**	**0,087**	Fisher exact	**5.44(0.66,60.6)**
Occupational therapy	**2**	**7**	**0,009**	Fisher exact	**14.46(1.44,259.45)**
Speech therapy	**2**	**6**	**0,032**	Fisher exact	**8.73(0.94,135.76)**
Median IQ/DQ					
Normal / Mild	7	2	0,184	Fisher exact	0.22(0.16,1.88)
Moderate/Severe	1	1	1,000	Fisher exact	1.35(0.02,117.49)
Special school	**2**	**5**	**0,159**	Fisher exact	**5.65(0.61,83.66)**
Ancestry					
European	0	4	0,021	Fisher exact	-
Indigenous Black	10	1	0,002	Fisher exact	0.03(0.001,0.43)
Mixed	2	4	0,331	Fisher exact	3.72(0.38,55.00)

Key: IQR = interquartile range; Peri- or postnatal complications: premature, respiratory distress, intrauterine growth retardation, hypoxic ischaemic encephalitis (HIE), trauma; Neuroimaging = MRI (in all) and/or CT: very minor non-specific changes; atrophy, asymmetry, subtle white matter changes supporting HIE; KD = ketogenic diet: trialled or offered to caregiver but declined, where trialled results partially or minimally responsive; ASD = autistic spectrum disorder; Special school: either placed or likely to be needed in future (once old enough). Areas of significance emphasised in bold text.

4. DISCUSSION

We have described the results of the first genetic study of DS in Africa. Despite the small cohort size, our findings carry significant implications for the diagnosis and management of children with DS in SA, and perhaps more broadly in Africa. Although the clinical features and genetic underpinnings of DS in our cohort were not novel, identification of nine patients carrying pathogenic or likely pathogenic *SCN1A* variants (41% of the cohort) and one female patient with a pathogenic *PCDH19* variant, confirmed that the genetic aetiology of DS patients re-diagnosed with FS+ was a likely consequence of the broad inclusion criteria of infantile-onset recurrent complex febrile seizures with normal prior development, which enhanced recruitment but also resulted in inclusion of the non-Dravet, FS+ phenotypes.

Statistical analysis of the clinical demographics highlighted the DS risk score and the age at seizure onset as the most useful clinical diagnostic markers for DS. The DS risk scoring system, devised by Hattori and colleagues, compared the clinical characteristics of Japanese Dravet and non-Dravet patients with seizure onset before one year of age [29]. In our cohort, the median score in the *SCN1A*-positive group (median score 9.00, range 8.00–11.00) was significantly higher than that in the variant-negative group (median score 5.50, range 3.75–6.00). This was in agreement with Hattori and colleagues, who proposed that a child with a score of six or higher was at an increased risk of DS and should undergo *SCN1A* testing [29]. However, the utility of this scoring system may be limited in the African context, as recognition of some of its diagnostic markers e.g., hemiclonic seizures (not usually seen in the earliest presenting period of DS), requires some experience in child neurology and epilepsy. In the African setting, where many of these children are initially seen by primary healthcare workers, straightforward clinical indicators are needed to prompt early referral to specialist centres. The significantly earlier median age of onset in the *SCN1A*-positive children in our

study (4 months, range 3–6 months), compared to the variant-negative group (12 months, range 8.75–13.25 months) was in line with previously published evidence showing that the onset of frequent and prolonged seizures before six months of age in an otherwise normally developing child, confers a high risk of progression to DS [31–33]. Thus, the age of seizure onset presents and uncomplicated early clinical indicator and an easily implemented specialist referral criterion that could enhance early detection of DS in resource-limited settings.

The low number of *SCN1A*-DS among the indigenous black children in our cohort (only one of 10), prompted a clinical reassessment, resulting in a revised diagnosis for nine patients, the majority of whom fell into the FS+ category. These children were more likely to be based in poorer socioeconomic settings, often with multiple healthcare challenges and at risk of recurrent infections, placing this group at an increased risk of recurrent FS. The results of our study therefore emphasise the importance of genetic testing not only for the variant-positive patients but also the patients in whom negative test results precipitate clinical reassessment and a revised diagnosis. Investigators in a recent epidemiological study of DS in the United States (US) found that clinical DS in the US occurs at an incidence of 1 in 15 700 births and is more than twice as common as previously reported [34,35]. The US study also reported a higher incidence of *SCN1A*-associated DS (1 in 20 900 births), compared to the European estimates ranging from 1 in 22 000 to 1 in 41 000 [13,36]. Applying the US DS incidence (1 in 15 700) in SA where 969 415 live births were recorded in 2016 [37], theoretically translates into approximately 62 new DS cases in a single year. Most will not have a diagnosis at the time of publishing this study and none (or very few in private healthcare), would have had access to genetic testing. In the Western Cape region of SA, the majority of children diagnosed with clinical DS are eventually referred to the Epilepsy Clinic at the RCWMCH. At the time of recruitment, only about 30 DS/possible DS patients were referred to the Epilepsy Clinic over a period of approximately 10 years. Using the regional birth registration figure of 106 599 in 2014 [38], one might extrapolate that approximately 7 new DS patients should be referred to the clinic each year (using the US DS incidence figure). It is therefore likely that many DS patients in the Western Cape and across SA are not diagnosed or managed appropriately. Most of these children could now be clinically recognized using the age of onset and the DS risk scoring system [29] and referred for genetic testing.

Our findings are new and especially useful in the African context, where genetic epilepsy research is limited and diagnostic testing is not available. The correct diagnosis and treatment of DS and other epileptic encephalopathies may be achieved months or years after initial presentation, if at all. The DS risk score and age of onset of prolonged febrile seizures before 6 months of age present quantitative low-cost criteria to identify patients most at risk of DS and who are likely to benefit from genetic testing [29,31]. Whilst the scoring system may be more relevant to patients with established DS beyond the early febrile stage, the age at seizure onset is a simple clinical marker for early DS. In the poorest and most remote rural regions, such simple diagnostic tools can assist in the choice of treatment and alert to the need for specialist consultation and genetic testing. As a direct result of this study, the Epilepsy Clinic at the RCWMCH now has nine patients with confirmed *SCN1A*-associated DS, and one patient with *PCDH19*-related epilepsy on its records. These findings highlighted the sometimes underestimated diagnostic precision of an informative

genetic test result in a child with possible DS. Anecdotally, the appreciation of “diagnostic closure” was strongly expressed by the parents and clinicians alike, also emphasizing the role of genetic counselling. Understanding the cause of the disease in a child brought about a sense of relief, acceptance and a more focused approach to care. Whilst the management typically followed the available interventions recommended for DS, including valproate and clobazam, following the genetic confirmation parents were more committed to accessing stiripentol and trials of the ketogenic diet. The study outcomes also presented a potential focus for future research towards identifying the causes of genetic and clinical heterogeneity in the “variant-negative” patients of our cohort.

5. CONCLUSION

DS is, arguably, the most extensively studied DEE [6] and also one of the most clinically challenging epilepsy syndromes. The intractable seizures, multiple co-morbidities and constant threat of premature mortality profoundly affect the quality of life of the children and their families. A better outcome can be achieved with appropriate intervention and more targeted therapy at an early stage of the disease, reflecting an example of precision medicine [39]. This is the first study investigating the genetic causes of DS in SA. Whilst conclusions drawn from small cohorts are generally viewed with caution, our results, as a snapshot of DS in the local population, confirm that *de novo SCN1A* variants are associated with disease in the majority of South African DS patients. Adding to the molecular findings, a significant outcome of this study was affirming the link between the DS risk score, the age at seizure onset and the presence of an *SCN1A* variant. In the poorly resourced African setting, observing these clinical signs of DS may go a long way towards embarking on the correct diagnostic course for DS and a better overall outcome. This also highlights the importance of raising awareness among healthcare practitioners of a possible genetic contribution to the seizure pathogenesis in their patients, thus beginning to bridge the significant epilepsy treatment gap in Africa.

Author contributions

AE: experiment design and analysis, article conception, drafting, collation of information, critical revision; GLC: experiment design, analysis and critical revision; RR: critical revision; SW: collation of clinical information; HCM: critical revision; JW: patient recruitment, article conception, clinical insights, critical revision.

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Conflicts of interests

The authors declare no conflicts of interests.

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4. MAIN STUDY

The following article, describing the outputs of this research study as a whole, is currently in press for publication in *Genetics in Medicine*. The supplementary materials referred to in this article are included in Chapter 6 of this thesis.

Journal: Genetics in Medicine

Title: *Precision medicine for Developmental and Epileptic Encephalopathies in Africa – Strategies for a Resource-limited Setting.*

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ABSTRACT (200 words)

Purpose: Sub-Saharan Africa bears the highest burden of epilepsy worldwide. A presumed proportion is genetic but this aetiology is buried under the burden of infections and perinatal insults, in a setting of limited awareness and few options for testing. Children with developmental and epileptic encephalopathies (DEEs) are most severely affected by this diagnostic gap in Africa, as the rate of actionable findings is highest in DEE-associated genes.

Methods: We tested 234 genetically naïve South African children diagnosed with/possible DEE, using gene panels, exome sequencing and chromosomal microarray. Statistical comparison of electroclinical features in children with and without candidate variants was performed to identify characteristics most likely predictive of a positive genetic finding.

Results: Of 41/234 children with likely/pathogenic variants, 26/234 had variants supporting precision therapy. Multivariate regression modelling highlighted neonatal or infantile-onset seizures and movement abnormalities as predictive of a positive genetic finding. We used this, coupled with an emphasis on precision medicine outcomes, to propose the pragmatic "Think-Genetics" strategy for early recognition of a possible genetic aetiology.

Conclusion: Our findings emphasise the importance of an early genetic diagnosis in DEE. We designed the "Think-Genetics" strategy for early recognition, appropriate interim management and genetic testing for DEE in resource-constrained settings.

INTRODUCTION

Epilepsy is one of the most common neurologic conditions, affecting approximately 50 million people worldwide.[1] Whereas most epilepsy research is conducted in resource-equipped countries, the highest burden of the disease is carried by Sub-Saharan Africa, ascribed to the high rate of infections, perinatal insults, traumatic brain injury, and under-resourced health care systems.[2] The stigma and misconceptions surrounding epilepsy in some communities often prevent the individuals and caregivers from seeking medical help. The resulting economical and psychosocial burden on the people and families living with epilepsy in low- and middle-income countries (LMICs) demand improved understanding and interventions. This public health imperative has been recognized through the development of the Intersectoral Global Action Plan on Epilepsy and Other Neurological Disorders. Among its global targets is a 50% increase in epilepsy service coverage by 2031 (from that in 2021) and development of legislation promoting and protecting the human rights of people with epilepsy in 80% of the member countries.[3]

A sizable proportion of epilepsy in Africa is presumed to be genetic, but the genetic architecture is largely undetermined within the context of minimal research and limited clinical testing. Genetic epilepsies and the associated syndromes are frequently missed or misdiagnosed and inappropriately treated.[4-6] The consequences are especially dire for children with developmental and epileptic encephalopathies (DEEs) in which early diagnosis and appropriate treatment are critical in mitigating the detrimental effects of ongoing seizures on the developing brain.[7] At the opposite end of the economic spectrum, in high-income countries (HICs), next-generation sequencing (NGS) gene panels, exome sequencing (ES), and chromosomal microarrays (CMA) are a routine part of diagnostic laboratory protocols, with genome sequencing (GS) having made the transition from research into the clinical laboratories in HICs.[8] Testing is mainly focused on the DEEs, in which the rate of actionable findings is the highest.[9,10] New knowledge of epilepsy-associated genes, genotype–phenotype correlations, and precision treatment approaches is swiftly translated into clinical practice.[11-14]

These economic disparities highlight not only the gap in service provision but also the lacking genetic epilepsy research in Africa.[5] In this article, we describe the genetic architecture of early-onset epilepsies in South African patients, using gene panels, ES, and CMA. Moreover, our detailed analysis of the electroclinical characteristics in individuals with and those without a detected genetic cause identified features that may be predictive of a positive genetic finding. We used this, coupled with an emphasis on actionable genes, to propose a pragmatic strategy for early recognition, testing, and precision treatment for DEEs in LMICs. We suggest that this approach, although different from that followed in HICs, may be effective in bridging the disparities in diagnosing genetic epilepsies in LMICs.

MATERIALS AND METHODS

Recruitment site

Most study participants were recruited from the Epilepsy Clinic at the Red Cross War Memorial Children's Hospital in Cape Town, a tertiary teaching hospital affiliated to the University of Cape Town and the main specialist care centre for paediatric epilepsy in Sub-Saharan Africa. Patients presented either directly to the

hospital or via specialist referrals for drug-resistant epilepsy assessments. The neurology service has a dedicated paediatric neurophysiology unit with access to video electroencephalogram telemetry and invasive monitoring by trained neurophysiology staff and accredited paediatric neurologists. All children with a recurrent seizure-onset age of less than 2 years undergo assessments for aetiology indicators and seizure semiology and managed for ongoing care. Standard assessments include exclusion of metabolic (urinary organic and amino acids, plasma ammonia, biochemistry, liver function, cerebrospinal fluid (CSF) protein levels, and paired CSF/plasma glucose and lactate levels) and structural pathology (computed tomography scan acutely, then brain MRI as part of initial assessment and typically by 2 years of age for optimal myelination), as well as assessment for immune-mediated encephalitis, if indicated. The clinic has access to ancillary services inclusive of rehabilitation and child development. Although there is capability of screening for most seizure aetiologies, access to genetic testing for epilepsy is currently lacking.

Study population

We recruited 234 genetically untested South African children between 2015 and 2019 with drug-resistant epilepsy and a diagnosis or suspicion of DEE, with no known infectious, metabolic, immune, structural (nongenetic) or other acquired cause. Although DEE is frequently associated with early-onset epilepsy (infantile or neonatal), we extended the age of onset to 8 years to ensure inclusion of possible late-presenting DEEs. A small number of children were recruited by affiliated neurologists in private practice. The study group comprised 122 males and 112 females, self-reported as indigenous Black African (*n* = 102), of South African mixed ancestry[15] (*n* = 90), Asian (*n* = 1), European (*n* = 17) or other/unreported (*n* = 24), broadly representing the population demographic of the South African Western Cape region. The group included 22 children clinically diagnosed with Dravet syndrome (DS), who were investigated in a pilot project.[16]

Clinical information collection and assessment

Information was collected through clinical assessment, review of patient records, parent/guardian interview, and/or clinician questionnaire, and was captured in a custom-designed Research Electronic Data Capture (REDCap) database[17] (Supplemental Table 1; Supplemental Note 1). To maintain consistency, data was captured by a team of the directly involved clinicians and molecular geneticists. Data that could not be confidently documented was preferentially excluded.

Genetic testing

DNA was extracted from peripheral blood using standard methods.

DEE-associated gene panel

All patients were tested using a panel of 71 DEE-associated genes using previously described methods[18-20] (Supplemental Note 2). Variants were filtered and prioritized for *de novo* and recessive variants. Only nonsynonymous, splice-site, and frameshift changes were assessed. Segregation analysis was performed when parental samples were available. Exon/gene-level copy number calling was performed as previously described and findings were confirmed using multiple ligase-dependent probe amplification or CMA.[21]

Variant classification followed published guidelines.[22] Microsatellite analysis was performed to confirm biological relationships.

CMA for genome-wide copy number variant detection

In total, 78 patients with no findings on the gene panel were selected for CMA analysis with a custom 4 x 180 K Comparative Genomic Hybridization array (Agilent Technologies) (Supplemental Note 3). Patient selection was based primarily on the availability of high-quality DNA. ClinGen CNV Pathogenicity Calculator was used for copy number variant (CNV) interpretation according to the published scoring metrics.[23]

Trio ES

A total of 20 patient-parent trios were selected for ES based on the absence of informative findings on the gene panel or CMA and availability of parental DNA. ES was performed as previously described using the Illumina HiSeq 2000 platform and the VCRome v.2.1 target-capture reagents (Roche Nimblegen).[24] We prioritized *de novo* and recessive variants for further pathogenicity assessment. Positive missense *z*-scores and probability of loss of function intolerance (pLI) scores were used as a criterion for non-truncating variant filtering in candidate genes.[25]

Clinical correlation

The available electroclinical information and response to antiseizure medications (ASMs) in each patient with a pathogenic/likely pathogenic (P/LP) variant were carefully compared with those described for the gene/variant to finalize the diagnosis and determine appropriateness of treatment (e.g., avoiding sodium channels blockers in *SCN1A*-DS). The functional effect (if known) of the variant was used to further refine options for precision treatment (especially relevant to the ion channel genes).[11]

Statistical analysis

The clinical characteristics assessed in the study population were described with descriptive statistics using R software (v. 4.1.1) and the R Studio interface. Comparisons were made between the characteristics in the following: (1) patients with candidate variants (any class) vs those without, (2) patients with candidate single/short nucleotide variants (SNV/indels) vs those without, and (3) patients with candidate CNVs and those without. In addition, multiple linear regression modelling was used to assess the association between selected clinical features (variables) and having an identified candidate variant. We included patients with P/LP variants and variants of uncertain significance (VUS) to avoid excluding individuals with VUS, which may be disease-causing, such as the patients in whom *de novo* occurrence could not be demonstrated in the absence of paternal DNA. The clinical variable selection was based on (1) statistical significance obtained in the preliminary data comparison ($P < .5$), (2) completeness of the data set (REDCap entry for most patients), and (3) obtainability through clinical examination.

RESULTS

Patient demographics and phenotypes

Of the 234 probands, 76 were recruited as singletons (probands), 108 proband-mother duos and 50

parent-proband trios. At least 1 episode of status epilepticus was recorded in 84 of 203 (41%) children (29 unknown). Global developmental delay (GDD) before seizure onset was recorded in 58 of 205 (29%) cases (31 unknown). Abnormal movements were noted in 39 of 234 (17%) and dysmorphic features in 26 of 234 (11%) cases. Autism spectrum disorder (ASD) was diagnosed in 30 of 234 (13%) children and attention difficulties in 21 of 234 (9%) (Supplemental Tables 2, 3 and 4). The overall median age at seizure onset was 8 months (interquartile range [IQR] = 3-18). Neonatal onset (0-1 month) was noted in 19 cases, infantile onset (2-24 months) in 165, childhood onset (>24 months) in 33, and was unknown in 17 children. The median time between the seizure onset and seeking medical assistance was relatively short for the neonates and infants (1 week and 3 months, respectively) but increased to 9 months in the childhood-onset group (Table 1).

During the course of the study, 14 of 234 (6%) children were clinically diagnosed with DEE due to other causes: acquired structural or infectious (*n* = 7), primary generalized dystonia (*n* = 1), tuberous sclerosis complex (*n* = 2), biotinidase deficiency (*n* = 1), thiamine deficiency (*n* = 1), Sturge-Weber syndrome (*n* = 1), and Moyamoya disease (*n* = 1). The possible underlying genetic aetiologies in these patients were not identified in this study owing to the panel gene content and the fact that only complete trios were selected for ES. We decided not to exclude these patients from the subsequent statistical analyses because they form part of a realistic referral base of the state specialist epilepsy service in South Africa.

The genetic architecture of early-onset epilepsy in South African children

Overall, rare genetic variants were detected in 51 patients: P/LP variants were identified in 41 of 234 (18%) children and 16 VUS were detected in 12 patients (Supplemental Table 5).

Sequence variants detected using a panel of 71 DEE-associated genes

P/LP SNV/indels were identified in 28 of 234 patients (12%) in 12 DEE-associated genes. Segregation analysis revealed *de novo* occurrence in 10 cases. Complete patient-parent trios were not available for the remaining 19 cases, but the available parents tested negative for the putative variants. *SCN1A* was the highest yielding gene (*n* = 13), followed by *STXBP1* (*n* = 3), *SCN2A* (*n* = 2), *KCNT1* (*n* = 2), and 1 variant, respectively, in *CACNA1A*, *CDKL5*, *CHD2*, *MECP2*, *PCDH19*, *SLC2A1*, *SCN8A*, and *SMC1A*. Five VUS were detected in *ATP1A2*, *KCNA2*, *SCN1A*, *SCN2A*, and *SCN3A*, respectively (Supplemental Table 5). More than two-thirds of the detected P/LP variants were found in ion channel genes.[20] All P/LP *SCN1A* variants were in patients with a clinical diagnosis of DS (*n* = 13) (Supplemental Table 1).

Genomic CNVs detected with CMA

P/LP CNVs were identified in 12 of 78 (15%) patients, with microdeletion/duplication syndromes detected and clinically confirmed in 6: 1p36 deletion syndrome (*n* = 1), Wolf–Hirschhorn syndrome (*n* = 1) Sotos syndrome (*n* = 1), 22q11.2 microduplication syndrome (*n* = 1), Angelman syndrome (*n* = 1), and Pallister-Killian mosaic syndrome (*n* = 1). Pathogenic whole-gene deletions were observed in 3 patients: a heterozygous *FOXG1* deletion in a child whose phenotype matched that of *FOXG1* deletion syndrome,[26] a *CDKL5* gene deletion in a female whose phenotype was consistent with *CDKL5* deficiency disorder,[27] and a deletion of *NBEA* at 13q13.3 in a child subsequently lost to follow-up; hence, no clinical correlation

Table 1. **Ages at seizure onset and the causative genes in the children with P/LP variants.**

Seizure onset	patients recruited	Patients with P/LP variants (SNV/indels +CNVs)	Patients with P/LP SNV/indels	Causative genes (number of P/LP variants detected)	Patients with P/LP CNVs	CNVs detected	Age 1st seizure: median months (IQR)	Age 1st seen for seizures: median months (IQR)	Age at molecular diagnosis: median months (IQR)
Neonatal (0–1 months)	19	6/19(32%)	5/19(26%)	*CACNA1A*(1), *CDKL5*(1), *SCN2A*(1), *STXBP1*(2)	1/19(5%)	16p13.11 deletion	0.75 (0.4 - 1)	1 (1 - 3.9)	108 (93 - 120)
Infantile (2–24 months)	165	29/165(18%)	22/165(13%)	*CHD2*(1), *KCNT1*(2), *NARS1*(1), *PCDH19*(1), *SCN1A*(13), *SCN8A*(1), *SLC2A1*(1), *SMC1A*(1), *STXBP1*(1),	7/165(4%)	22q11.21 duplication(1), 16p13.11deletion(1), 16p13.11 duplication (1), *FOXG1* deletion(1), 4p16.3p16.1 deletion(1), 5q35.2q35.3 deletion(1), 1p36 deletion (1).	6 (4 - 13)	9 (3 - 17)	108 (96 - 156)
Childhood (>2 y)	33	4/33(12%)	2/33(6%)	*SCN2A*(1), *MECP2*(1)	2/33(6%)	Pallister Killian Syndrome; 13q13.3 deletion (del *NBEA*)	35.5 (28.25 - 47.75)	44.5 (40 - 57.75)	144 (120 - 180)
Unknown	17	2/17(12%)	0/17 (0%)	-	2/17(12%)	Xp22.13(del *CDKL5*); 15q11.2-q13 abnormality (Angelman syndrome)	-	-	-
Total	234	41/234(18%)	29/234(12%)	-	12/234(5%)		-	-	-

SNV: single/short nucleotide variants; CNV: copy number variants; IQR: interquartile range.

was possible.[28] Heterozygous loss at the 16p13.11 susceptibility region was detected in 2 patients of which 1 was maternally inherited. Another patient had a maternally inherited 16p13.11 gain and a novel *KCNA2* missense VUS. The *KCNA2* variant was not found in his mother (who tested positive for the 16p13.11 gain); however, no paternal sample was available to establish/exclude *de novo* occurrence (Supplemental Tables 1 and 5).

ES in parent-child trios reveals rare genetic causes and novel candidate genes for the DEEs

De novo analysis identified candidates in 5 genes: *ANGPT1* (*n* = 1), *COBLL1* (*n* = 1), *GLUL* (*n* = 1), *NARS* (*n* = 1) and *PLPPR4* (*n* = 1) (Supplemental Table 5). Of these, only the de novo recurrent *NARS* variant fulfilled criteria for pathogenicity and the patient's clinical features aligned with the *NARS* neurodevelopmental phenotype described by Manole et al.[29] Analysis for autosomal recessive inheritance revealed compound heterozygosity for VUS in 3 genes (*UNC80*, *CELSR2*, and *APC2*) (Supplemental Table 5). To our knowledge, only *NARS* and *UNC80* genes have been definitively linked with phenotypes involving epilepsy. We engaged the GeneMatcher platform to connect with other investigators and learn more about the VUS detected in the other genes.[30] None have been resolved to date.

A genetic diagnosis affects clinical care and management

Actionable variants were detected in 8 genes (*CDKL5, KCNT1, PCDH19*, *SCN1A, SCN2A, SCN8A*, *SLC2A1*and *STXBP1*),[9] supporting precision therapy for 63% (26/41) of the individuals with P/LP variants and 11% (26/234) of the children overall[9] (Supplemental Table 1). ASM changes included dose optimization (e.g., carbamazepine) or revisiting motivation for access to restricted ASMs (e.g., stiripentol). Knowledge of the genetic aetiology also enabled a more targeted multidisciplinary team (MDT) care and better insights into prognosis, disease course and complications (Supplemental Table 1; Figure 1). Cascade testing of the at-risk or affected relatives was offered to the families of the patients with maternally inherited CNVs (22q11.21 gain, 16p13.11 loss, and 16p13.11 gain). In 2 of these cases, the mothers were mildly affected, which was only noted after testing the children. Prenatal analysis is now available to these mothers for potential future pregnancies.

Statistical analysis reveals specific clinical features more frequently associated with disease-causing SNV/indels and CNVs

Causative variants were identified and clinical syndromes were confirmed in 41 of 234 (18%) children in our study. Of these, the mean age at a molecular diagnosis was 108 months (IQR = 93-120) in neonatal-onset epilepsy group, 108 months (IQR = 96-156) in the infantile-onset group, and 144 months (IQR = 120-180) in the childhood-onset group (Table 1). Patients with neonatal-onset epilepsy had the highest proportion of P/LP SNVs/indels (32%, 6/19), followed by patients with infantile onset (18%, 29/165) and patients with childhood onset (12%, 4/33). CNVs were detected in approximately 5% of the children in each age-of-onset group (Table 1). However, it must be noted that in reality this figure is likely to be higher because only 78 children were CMA-tested.

There were no significant differences between the seizure types recorded in individuals with and individuals

Case 1	Case 2	Case 3
Variant: *SCN2A*:c.5836A>G, p.(Lys1946Glu), VUS (LP if de novo)	**Variant: *SCN2A*:c.656T>C, p.(Phe219Ser), LP; GoF**	**Variant: *SCN2A*:c.4551+1G>A, rs527688117, P, LoF**
Patient: a nine-year-old Indigenous Black African boy, referred for untreated seizures. **Seizure semiology**: one-year history of unprovoked frontal seizures with loss of awareness, eye rolling, starring, tonic posturing and clonic movements of 2-minute duration, 2-3/day; aggressive post-ictally. **History:** previously functionally and cognitively intact; marked behavioural and intellectual regression and ASD after seizure onset; episodes of confusion, headaches, combative outbursts, occasional gait unsteadiness; limited verbal communication, unable to remain in mainstream education. Extensively investigated to exclude structural, toxicological, post-infective and autoimmune aetiologies. **EEG:** low background with right pre-frontal sharp and slow waves; defuse discharges from both hemispheres but mostly over the frontal regions; seizure activity predominantly from the right frontal regions on ictal video EEG. **Treatment and outcome:** seen by a traditional healer for a year before accessing conventional healthcare; background of a rural setting with significant social challenges. No response to VPA; some improvement on adding carbamazepine post-genetic finding; poor adherence confirmed with subtherapeutic levels; adherence improved post counselling, seizures reduced to one 30-second seizure/2 weeks. Persisting learning and behavioural issues. **Comment:** this case is typical of many children in the socioeconomic circumstances of the rural African setting, whose care is compromised by delays, deferment to alternative health providers, poor ASM compliance and limited health and educational support. *De novo* occurrence could not be established in absence of paternal DNA. Nonetheless, the genetic finding pointed in a direction of an ultimately successful treatment option. Actioning a VUS however, requires careful consideration of possible risks and benefits, ultimately best done as part of a MDT consultation.	**Patient:** a five-week-old Mixed Ancestry boy, referred for treatment-resistant seizures. **Initial seizure semiology:** onset at three woa; regular jerking movements in clusters of 3-4/episode; sudden extension of the arms (predominantly right) and associated deviation of the mouth and eyes; 5 – 10 second duration, some crying afterwards. **History:** a complex antenatal course; significant recreational drug exposure throughout the pregnancy; born at term via caesarian section (Apgar: 6 at 1 min, 9 at 5 min); treated with antibiotics for presumed neonatal sepsis; poor suck in the first week; increased tone noted subsequently. Cerebral palsy (GMFCS 5) and severe GORD, fed via a gastrostomy tube. Placed in a care facility due to social concerns. One older, developmentally normal sibling. **EEG:** markedly abnormal intermittent ictal events with corresponding abnormalities, predominantly left temporal spikes; low voltage followed by slowing and sharp wave activity; independent and bilateral slow and sharp wave activity. **Brain Imaging:** CT: decreased myelination with left parietal lobe pachygyria; MRI (3-month): low white matter volume, abnormal signal intensity in the periventricular regions, age-appropriate myelination, thin corpus collosum; no features of pachygyria, consistent with moderate to severe hypoxic-ischemic encephalopathy (HIE). **Treatment and outcome:** multiple, daily focal seizures on phenobarbital; no improvement with added VPA. Marked improvement with high dose carbamazepine, after identification of the *SCN2A* variant, at three yoa. **Comment:** antenatal drug exposure was the assumed cause of this child's illness, highlighting the importance of considering a genetic cause in early-onset, drug-resistant epilepsy, even when the patient's history suggests otherwise. The electroclinical features, age at seizure onset and the marked improvement on carbamazepine treatment were all typical of a *SCN2A*-DEE with an underlying GoF variant.	**Patient:** 13-year-old Indigenous Black African girl for drug-resistant seizures. **Initial seizure semiology:** onset at 4 yoa; clusters of GTCS, 5 – 9/day, escalating in duration until medical intervention required; pre-ictal altered behaviour, guttural sounds and eye deviation; GDD from infancy, soft dysmorphism: flat nasal bridge, hypertelorism, small ears, full lips and bulbous nose; scoliosis; aggressive and hyperactive, ASD diagnosed at 2 yoa. **Subsequent history:** Profound, static GDD (DQ 40). Short, early morning tonic seizures, 3/week. **EEG:** Background of parieto-occipital region sharp waves, evolving into left parietal discharges and occasional 8-second runs of sharply contoured activity from the left frontal region, without clinical accompaniment. Several 30-second probable electrical events with posterior onset, more marked from the right but evident bilaterally, starting with a burst of sharply contoured activity at 9-12Hz frequency, then fluctuating between 4Hz and 9 Hz. Not typical of a seizure but with a clear change in the background activity. The full extent of the activity possibly undetected by the surface electrodes. No change in clinical activity. **Neuroimaging:** normal CT and MRI **Treatment and outcome**: trials of lamotrigine, leviteracetam, carbamazepine and clobazam ineffective. Fewer events with no hospital intervention on a combination of VPA, carbamazepine and phenobarbital. However, seizures remain drug-resistant. **Comment**: an earlier molecular diagnosis of *SCN2A*-DEE, could have consolidated the condition and supported a more targeted treatment approach. The child's mother was counselled about the genetic finding but was reluctant to "interfere" with the ASMs. This is a sad reflection of the delayed diagnosis and the battle-weary family accepting the current refractory situation as good-enough.

GMFCS 5: Gross Motor Function Classification System Expanded and Revised level 5; GORD: gastro-oesophageal reflux disorder; GoF: gain of function, LoF: loss of function; MDT: multidisciplinary team; yoa: years of age; VPA: sodium valproate; woa: weeks of age.

Figure 1. **Case Box descriptions of three patients with *SCN2A* variants with different variant types, ages of seizure onset and responses to treatment, demonstrating the relevance of the functional effect of the underlying variant to precision treatment.**

without P/LP variants, stratified per age at seizure onset (neonatal, infantile, and childhood). The 3 most frequently noted seizure types in all groups were generalized tonic-clonic, focal, and myoclonic seizures. Febrile seizures were prevalent among children with infantile-onset epilepsy, almost all diagnosed with DS (Supplemental Figure 1, Supplemental Table 1). Dysmorphism and GDD before seizure onset were noted in more than half of the patients with CNVs compared with less than a third of the patients with SNVs or no candidate variants. ASD and attention difficulties featured prominently among individuals with SNV/indels (20%). The SNV/indel group also had the greatest proportion of patients with movement abnormalities (47%) and the youngest age at first seizure (median 5 months [IQR = 2-9]). Structural brain anomalies were prominent in the CNV group (Figure 2, Supplemental Tables 2, 3 and 4).

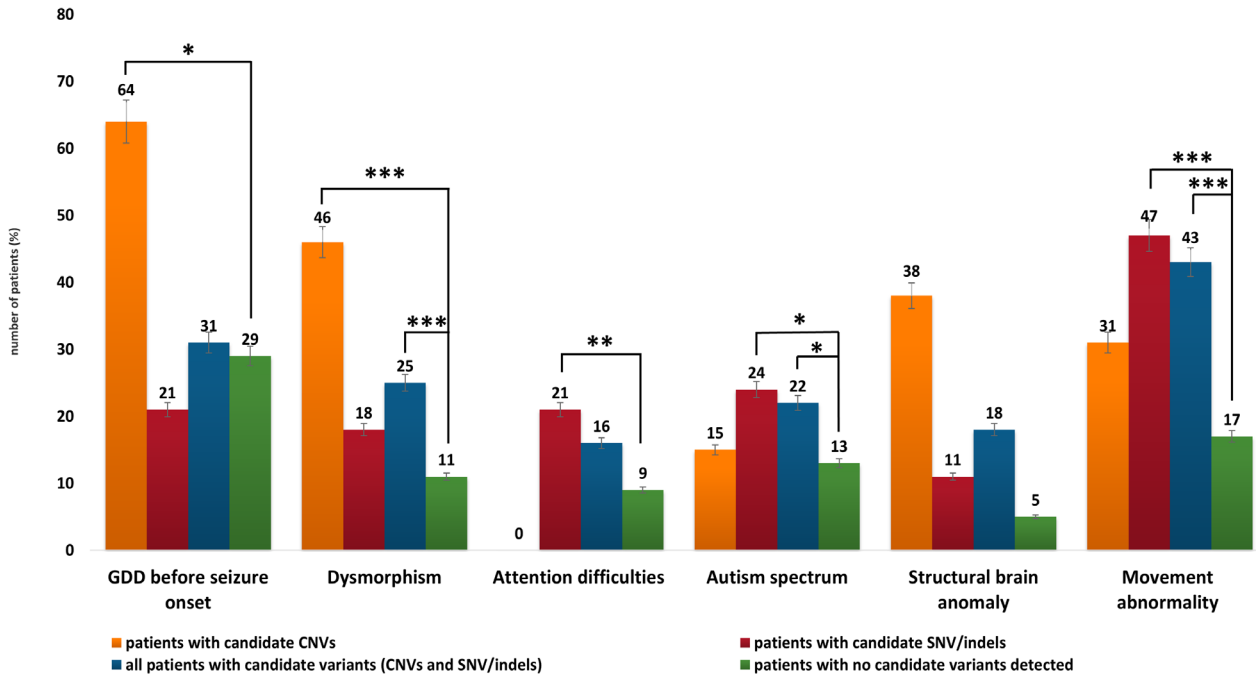

Figure 2. **Statistical summary of selected clinical features in patients with candidate genetic variants.** The number of patients with a variant in each class (i.e., CNV, SNV/indel, CNV and SNV/indel combined,) and the specific clinical feature are shown. For instance, 60% of the patients with a detected CNV had GDD before seizure onset, 21% of the patients with a detected SNV/indel had GDD before seizure onset, etc. No line/star: $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Multivariate logistic regression analysis suggested associations between movement abnormalities (odds ratio [OR] = 6.08, 95% CI = 2.49-15.2) and attention difficulties (OR = 3.96, 95% CI = 1.24-12.3) with the presence of a candidate SNV/indel (Figure 3, model A; Supplemental Table 6), whereas structural brain anomalies achieved significance among patients with CNVs (OR = 22.8, 95% CI = 3.25-181) (Figure 3, model B; Supplemental Table 7). These features were also noted in the combined variant model (SNV/indels and CNVs) (Supplemental Figure 2*;* Supplemental Table 8*)*. ASD, dysmorphic features, GDD, and age at first seizure did not show significant associations with the presence of a candidate variant in the multivariate regression model but did achieve statistical significance in the initial statistical analysis ($P<0.05$) (Figure 2; Supplemental Tables 2, 3 and 4).

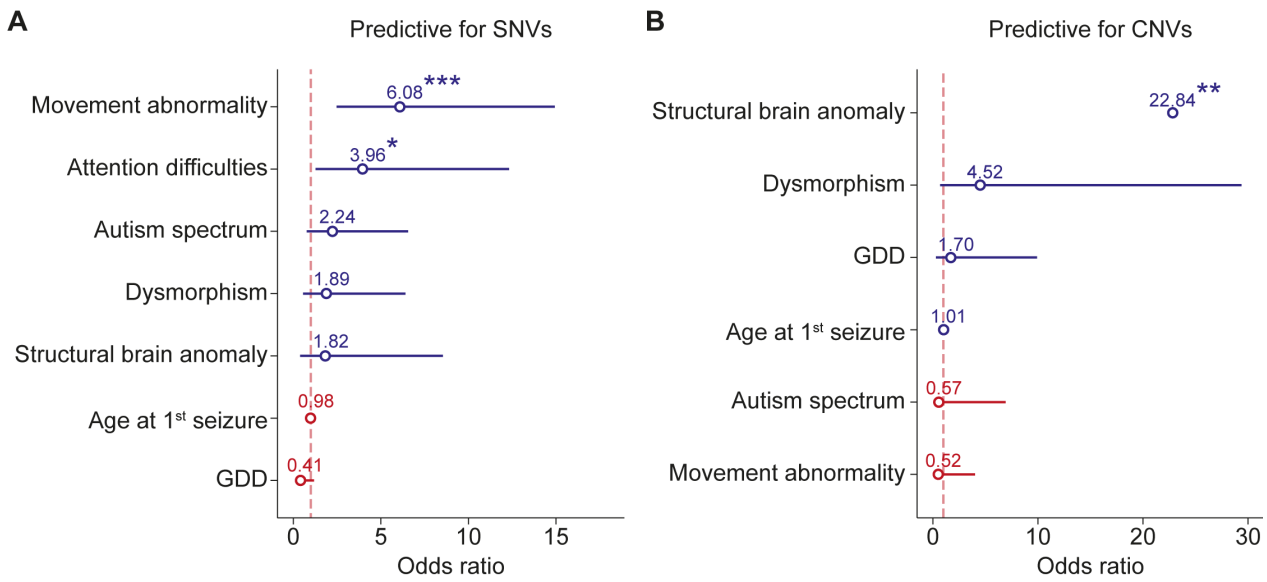

Figure 3**. Multivariate Logistic Regression Analysis for assessment of association between selected clinical features in patients with and without an identified genetic aetiology.** (A) SNV/indels: significant associations were observed with movement abnormalities and attention difficulties (B) CNVs: significant associations were observed with structural brain anomalies. *p≤0.05; **p≤0.01 and ***p≤001.

A Think-Genetics decision tree for an early diagnosis of a suspected genetic epilepsy/DEE in the LMICs

We used 3 facets of our results to develop the Think-Genetics (TG) decision tree, designed to help clinicians at patient-entry in resource-constrained settings to recognize a possible genetic epilepsy/DEE and initiate a process of MDT consultation and genetic testing (Figure 4).

1. Clinical features associated with the presence of P/LP variants in our study: neonatal or infantile-onset, drug-resistant seizures are most likely to have a currently identifiable genetic cause.[31] We identified movement abnormalities (stereotypical hand and arm movements, gait abnormalities, eyelid myoclonia), attention difficulties, ASD and dysmorphic features as additional strong indicators of a genetic aetiology. Structural brain anomalies were excluded from this model, despite the strong statistical association with CNVs (Figure 3), because the description included nonspecific features such as brain atrophy and thinning corpus collosum, making it a relatively weak criterion for a genetic aetiology. Moreover, structural anomalies are identified through imaging and thus cannot be described as a simple diagnostic marker.
2. Precision medicine implications: initial testing with a starter DEE panel is more affordable and easier to set up locally than ES or GS. Almost all P/LP SNV/indels in our study were detected in 12 genes included in the NGS panel, listed among the 20 top-yielding DEE-associated genes in published large-scale studies.[9,32,33] Therefore, initial testing with a small panel of genes selected on the basis of clinical actionability and frequency of association should solve a good proportion of the DEE cases with possible options for precision treatment (Supplemental Note 4). This has been previously suggested by other investigators.[34,35]

3. Choice of initial genetic testing modality: we suggest CMA testing as the initial genetic investigation for patients with dysmorphic features and GDD before seizure onset, because these were prominent in patients with CNVs in our study. If negative, NGS-based testing for SNV/indels should follow. Conversely, patients with neonatal/infantile-onset seizures and normal prior development should be tested using NGS first (starter panel) followed by CMA if negative. MDT consultation should precede further testing (ES or GS), because additional information may come to light, redirecting the diagnostic focus away from a genetic cause.

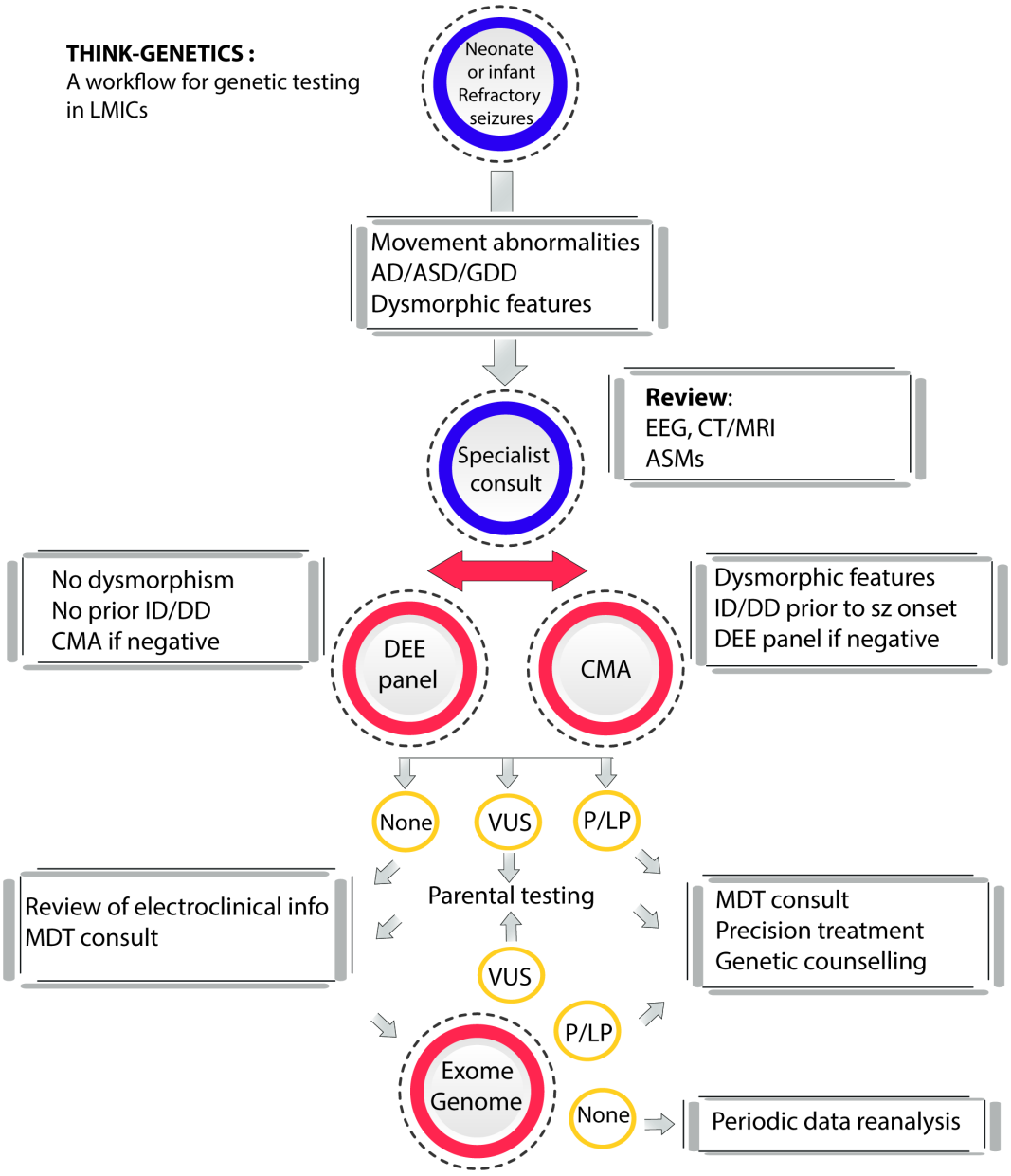

Figure 4. **The "think-genetics" (TG) decision tree for recognition and genetic diagnosis of DEE in a low-income setting.** The gene panel used may be small, incorporating only genes with implication for precision treatment and those commonly implicated in DEEs (Suppl. Note 4). Ideally, individuals without a diagnosis after initial testing would undergo ES, though this is limited and available only to a fraction of patients clinically in our LMIC setting. AD: attention difficulties, ASD: autism spectrum disorder, ASMs: antiseizure, medications CMA: chromosomal microarray, GDD: global developmental delay, GS: genome sequencing, ID/DD: intellectual disability/developmental delay; MDT: attention difficulties, VUS: variant of uncertain significance.

DISCUSSION

The findings of our study show first-hand that access to genetic testing for DEE in the under-resourced, tertiary hospital setting in South Africa carries similar clinical and economic benefits to those described in the HICs,[9,36] justifying the cost of NGS and CMA.

The yield of P/LP variants in our study (18%) was lower than anticipated, based on our panel size and published reports of similar testing in DEE, reporting diagnostic yields of 20% to 45%.[32,35] This is likely because of the broad inclusion criteria and complexities of the local patient referral system. However, our findings did mirror international conclusions in terms of the diagnostic yield relative to the age at seizure onset. Most of the P/LP variants in our study were identified in children with neonatal and infantile-onset seizures (35/41, 85%) (Table 1).[31] The four top-yielding genes in our panel (*SCN1A*, *STXPB1*, *SCN2A*, and *KCNT1*) accounted for approximately half of the solved cases (20/41, 49%) and two-thirds of the detected P/LP SNV/indels (20/29, 69%), also in keeping with the published DEE research.[34,37] Conspicuously absent from our group were pathogenic variants in *KCNQ2*, which ranks among the five top-yielding genes in large-scale studies and is linked to a phenotypic spectrum spanning the benign familial and the severe *de novo* DEE phenotypes.[9,32,33] The reasons for this bear further investigation but may be related to our relatively small cohort size and possible patient exclusion on presumption of infection or hypoxia as the most common local aetiologies of neonatal seizures.

Only one P/LP variant (*NARS*) was identified on ES of 20 panel-negative proband/parent trios. ES testing of the entire cohort would no doubt yield additional findings and we do not question its many advantages. However, gene panels are less expensive than ES or GS, require less skill and computational power to analyse, and yield fewer VUS and almost no incidental findings. This is relevant in the African context, because of the limited publicly accessible data on allele frequencies in African populations, especially in Sub-Saharan Africa. *De novo* occurrence as the major criterion for variant interpretation in DEEs[38,39] is often difficult to establish in the African setting, with its high prevalence of the so-called “orphan households”[5,40]. Therefore, we propose a small panel of 32 DEE genes as a pragmatic and high-yielding first-tier genetic test for DEEs in LMICs. If negative, this would be followed by ES (if accessible) if there is still an indication and budget for further testing (Figure 4). Importantly, whilst much of the genetic testing in this study was performed in the United States, the work is being translated into service, with a DEE gene panel in the process of validation within the diagnostic laboratory at the University of Cape Town and the South African National Health Laboratory Service. Although CMA analysis is available, ES and GS currently remain accessible to only a handful of South African patients in the private health sector or through research.

The proportion of the detected P/LP CNVs was high (12 out of 78 CMA-tested patients, i.e., 15%), mostly diagnostic of classic microdeletion/duplication syndromes (Supplemental Tables 1 and 5). In a well-resourced setting, these patients would have been tested with an early diagnostic CMA and excluded from the study. Our patients, however, were referred to the epilepsy service for seizures as the initial presentation or major concern and their care focused on acute needs, such as seizure control, development, respiratory, or feeding problems. We ascribe the late diagnoses to the subtle presentation or incomplete penetrance in

some cases. We did not exclude these patients from subsequent analyses because our goal was to assess a real-life patient population in a paediatric epilepsy clinic in South Africa. The CNV detection highlighted the need to review the current local referral and clinical reassessment protocols, incorporating CMA testing for epilepsy-plus phenotypes, especially those with intellectual disability and dysmorphism.[41]

Of the 41 children with P/LP findings, 26 had variants with treatment (Supplemental Tables 1 and 5). The growing understanding of the mechanisms of variant pathogenicity and effect on ASMs, with treatment recommendations or contraindication based on the underlying genotype, is perhaps the most compelling reason for availability of clinical genetic testing, especially for the DEEs.[9,42] The relevance of the functional effect of the underlying variant to the choice of treatment was demonstrated in our study in three patients with *SCN2A* variants, each with a different variant type, age of seizure onset, and response to treatment (Figure 1; Supplemental Table 1).[9,11] These cases are an exciting example of precision treatment, often considered beyond the reach of health care in Africa.

Compared with similarly presenting patients in the HICs, the children in our study obtained their genetic diagnoses late, years after the onset of epilepsy (Table 1). The median age at molecular diagnosis among the infantile-onset group was 108 months (IQR = 96-156) compared with the median age of 6 months (IQR = 4-13) at seizure onset. However, even the late genetic diagnoses led to treatment adjustments and positive changes in the lives of the patients and the families. The value of the diagnostic closure, even in absence of direct implications for treatment is vastly underestimated, especially in resource-constrained settings where genetic testing may be viewed as nonessential. Anecdotal feedback from the recruiting clinicians conveyed the profound relief and gratitude from the parents, for whom knowing the reason behind their child's illness brought closure and focus on the way forward. The clinicians were also grateful to have the answers and excited to gain new and useful knowledge. Few - if any - other research studies in that environment to date have elicited an equally positive response and ongoing enquiry from the families and the clinicians.

One may question the economic feasibility of weighing down the hospital budgets in resource-constrained settings with an expensive genetic investigation, which carries a realistic pick-up rate of less than 40%, even in a well-phenotyped and appropriately referred patient population. However, the overall expenses incurred during the so-called diagnostic odyssey are exceedingly high and the downstream savings carried by a molecular diagnosis outweigh the cost of testing.[10,43] At the time of writing this manuscript, the combined cost of a gene panel and a CMA in South Africa amounted to approximately 20,000 ZAR (~US$1200). This is similar to the cost of a single brain MRI, often ordered more than once during the course of the child's illness as a standard investigation for DEE. Added to this are the costly metabolic screens (~US$200 per test) and hospital admissions.

In the South African daily clinical practice, recognition of possible signs of a genetic epilepsy is complicated by the layering effects of tuberculosis, HIV, parasitic and other febrile illness, perinatal insult, poor nutrition and other complications of the socio-economic circumstance. Based on our findings, we designed the simple

TG decision tree that could guide the primary care clinician in Africa in recognizing signs of a possible genetic aetiology among the prevalence of seizures with an acquired cause. These patients could then be triaged correctly and much earlier on for specialist consultation, correct intervention, and, ideally, genetic testing. The few analysable variables and relatively small cohort size limited our ability to draw decisive conclusions from the multivariate analysis in this study. Nonetheless, our findings suggest that in addition to neonatal- or infantile-onset drug-resistant seizures, movement abnormalities, attention difficulties, ASD, dysmorphic features, and GDD are strong additional indicators of a genetic aetiology, regardless of age at seizure-onset (Figures 2 and 3).

Conclusion

To our knowledge, this is the first published study of the genetic underpinnings of patients with DEE in Sub-Saharan Africa. An important characteristic of our study group, compared with the investigations conducted in well-resourced settings, was the absence of selection bias imposed by prior genetic testing. The convoluted diagnostic history of many children in our study, reflects the time delays between seizure onset, seeking medical help, and eventual correct diagnosis and treatment. Our TG decision tree may help to limit such delays by simplifying decision-making and setting the clinician at patient entry on a correct diagnostic course from as early as the first visit.

Data availability

Tables listing deidentified clinical information, genetic variant details, statistical summaries and regression analyses are included in the Supplemental Information. Deidentified raw data may be available on request from the corresponding author.

Conflict of interest statement

The authors have declared that no conflict of interest exists.

Author contributions

Conceptualization: A.I.E., H.C.M., R.R., J.M.W., G.L.C.; Data curation: A.I.E., G.R., M.W., J.M.W.; Formal analysis: N.T., M.C.A., J.D.C., J.G., M.J.B.; Funding acquisition: A.I.E., R.R., J.M.W., G.L.C.; Investigation: A.I.E., M.W., G.R., M.C.A., E.E.A., A.R., J.M.W., G.L.C.; Resources: H.C.M., J.M.W., G.L.C.; Software: R.J.B., J.D.C., J.G.; Supervision: R.R., J.M.W, G.L.C.; Visualization: A.I.E., R.J.B., G.L.C.; Writing-original draft: A.I.E.; Writing-review & editing: A.I.E., N.T., R.J.B., H.C.M., R.R., J.M.W., G.L.C.

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5. DISCUSSION

For the purpose of this discussion, the article in press for publication in *Genetics in Medicine* (GIM), describing the main study findings will be referred to as the "**GIM paper**". The article published in *Seizure*, describing the DS pilot study will be referred to as the "**DS paper**". The review article published in *Frontiers in Neurology* addressing the challenges surrounding genetic epilepsy diagnosis, research and testing in Africa, will be referred to as the "**FIN paper**". This Discussion aims to complement the publications by addressing the points not discussed or highlighted in their content.

5.1. Overview

This body of work describes the findings of an investigation of the genetic causes of early-onset epilepsies in SA patients and explores the challenges and opportunities for meaningful research and genetic service for epilepsy in Africa. It also puts forward a proposed strategy for early genetic testing and precision treatment for DEEs in a resource-limited setting.

An important characteristic of this study group, compared to many epilepsy studies conducted in well-resourced settings, was the absence of selection bias imposed by prior genetic testing. Genetically 'naïve' patients were recruited in a real-time clinical setting, using age of seizure onset (<8 years) and refractory epilepsy of unknown aetiology with developmental plateauing or regression as the main inclusion criteria. This broad approach was taken to avoid missing late-presenting DEEs and children whose early seizures may have gone undiagnosed or unnoticed within the socioeconomic and resource-constrained living conditions of many SA patients seen in the state hospitals. However, the majority of the children were under 2 years of age at seizure onset. None of the children had had any prior genetic testing, mainly due to its limited availability locally. Overall, likely/pathogenic SNVs and CNVs were identified in 17.5% (41/234) of the study group tested with a combination of a targeted gene panel, CMA and exome sequencing. Of these, 63% (26/41) had implications for treatment (Figure 5).

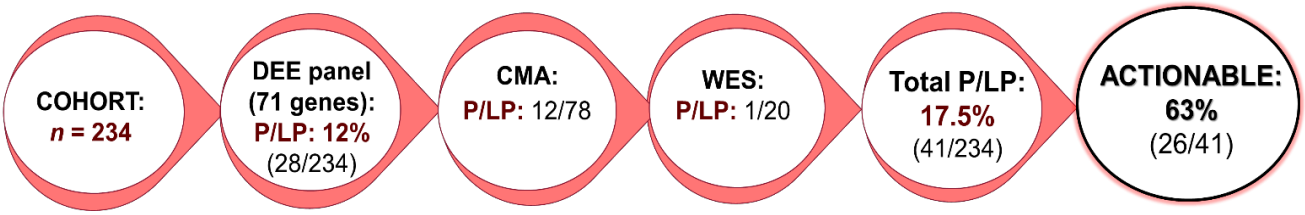

Figure 5. **Genetic testing in the study group: workflow and outcomes.**

Despite the limited knowledge of genetic underpinnings of epilepsy in African populations, in view of the mainly *de novo* nature of the DEEs and taking into account the methodology employed, the genetic findings were expected to be similar to those in the published DEE cohorts. It was therefore not surprising that the ion channels genes harboured two thirds (19/29, 66%) of the detected P/LP Single/short Nucleotide Variants (SNVs)/indels, with the highest yield in *SCN1A*, all in patients with DS (13/29, 45%) (Suppl. Tables 1 and 2, GIM paper). Nine of the DS patients were part of the initial pilot study published in *Seizure*, which was the first genetic study of DS in African patients. It confirmed that *SCN1A* is, as expected, the main gene implicated in DS in Africa and

highlighted the onset of refractory febrile seizures before 6 months of age, as an important diagnostic predictor for DS (181).

As noted in the DS paper, NGS testing for the DS pilot was performed at UCT on the Ion Torren platform (Thermofisher Scientific, USA) and then again on the Illumina HiSeq™ instrument in the USA, confirming the findings and validating the local process (181). Publishing this pilot highlighted the value of raising awareness of a genetic aetiology and the relevance of genetic testing, with an increase in the detection rate of DS by the local entry-receiving clinicians (emergency staff) since the publication.

The electroclinical information recorded for the LP/P variant-positive patients in the GIM paper appeared to fit within the phenotypic spectra of the underlying genes, as did, for the most part, the response to ASMs. This allowed the managing clinicians to finalise the diagnoses and assess the appropriateness of treatment, especially for the children with channelopathies (Suppl. Table 1, GIM paper). Almost two thirds (26/41, 63%) of the children with P/LP findings had variants with treatment implications, such as the contraindication for the use of channel blockers or beneficial effects of the ketogenic diet and would have benefited from earlier testing. For example, carbamazepine had been trialled (among other ASMs) on most of the children with DS, and a worrying number also received lamotrigine (Suppl. Tables 1 and 5). This would not have been the case, had they been tested sooner, potentially influencing the severity and progression of their disease. Certain ASMs, such as stiripentol, are not freely accessible in the SA public sector. A special motivation and a definitive diagnosis of DS is required, which would have been greatly expedited by a molecular confirmation. The choice of treatment can be further refined with the knowledge of the functional effect of the specific P/LP variant (loss or gain of function) (157). This was effectively illustrated by the three patients with *SCN2A*-DEE with varying ages of seizure onset and responses to treatment, as described in detail in the Figure 1 Case Boxes in the GIM paper. These cases are an exciting example of the clinical utility of genetic testing and application of precision treatment, demonstrably achievable within the context of healthcare in Africa. Such findings promote awareness and inspire further research.

The value of the remaining positive findings (those with no or less profound treatment implications) lay in establishing the cause of seizures in the child, bringing an end to the diagnostic odyssey for the patient, family, as well as the clinician. *STXBP1* was the second most commonly implicated gene in the study, which was largely consistent with the detection rates for the genes reported in international DEE cohorts (182–184). The phenotypic features and disease course reported in the three patients with *STXBP1*-DEE, aligned with those published for this extensively studied gene and DEE (185). All three patients (EE22, EE26 and EIEE19) had initially normal development, with two (EE22 and EE26) suffering severe regression after the onset of seizures (Suppl. Table 1, GIM paper). Stereotypical movements, one of the most common features of *STXBP1*-DEE were recorded in all three patients(186). The DEE in one case (EIEE19) had been ascribed to the HIE and CP, demonstrating the importance of not automatically discounting a genetic cause in the presence of other possible causes. Whilst these patients have responded well to their respective treatment regimens, periods of seizure freedom are well documented in *STXBP1*-DEE (186) and levetiracetam with its superior effect on seizures and movement disorders in *STXBP1*-DEE remains at option, if required (17).

The well-described genetic and phenotypic overlap of the DEEs was also demonstrated in this study group. Examples included a child with a P/LP *CHD2* variant of undetermined origin, initially suspected of having DS. *CHD2*-related epilepsy has been compared to DS, though few *CHD2* patients have febrile seizures (24,187), which were also not recorded in this patient. The patient had features typically seen in *CHD2*-related epilepsy; the atonic-myoclonic-absence seizures, eyelid myoclonia [recently also described as a distinctive feature of DS(188)] and behavioural problems. Another finding in the DS pilot study was a *de novo PCDH19* nonsense variant in a female patient (EE11) subsequently diagnosed with *PCDH19*-related DEE, previously known as the Epilepsy and Mental Retardation limited to Females (EFMR). *PCDH19*-related epilepsies display the male-sparing X-linked inheritance, where heterozygous females are affected but hemizygous males are unaffected. Affected somatic mosaic males have also been reported (189). *PCDH19* encodes protocadherin 19, a calcium-dependent cell-cell adhesion molecule that is highly expressed in the CNS. The specific pathomechanism of cellular interference has been demonstrated in mouse models, where investigators have shown that cell-cell interactions are disrupted in heterozygous cell populations (wildtype *and* altered *PCDH19*-containing cells*),* causing abnormal segregation during brain development. This abnormal segregation is not seen in homozygous females or in male mice lacking *PCDH19*, which is consistent with what is observed in humans (190). The patient's paternal grandmother was said to have epilepsy and it was therefore expected to find the variant in the unaffected father, which was not the case. The grandmother's DNA was unavailable for testing but her seizures were said to have started in adolescence and she was currently on medication and seizure-free. It is therefore likely that the grandmother's seizures have a different aetiology. This case illustrates that a family history of epilepsy does not necessarily equate with a genetic risk, as shared environmental risk factors may contribute towards the recurrence of seizures or epilepsy among multiple individuals in the same family (191).

Findings in X-linked genes also included *CDKL5* and *SMC1A*. *SMC1A* DEE can mimic the *PCDH19*-related disorder, with prolonged clusters of drug-resistant multiple focal and generalised seizures. This was observed in a female patient (EE99) with a splice *SMC1A* variant, who presented at 16 months with clustering GTCSs, evolving into monthly focal seizures (and no dysmorphic features or other hallmarks of Cornelia de Lange Syndrome). Her development was mildly delayed prior to seizure onset with no language, seizure-associated neuroregression and stereotypic hand movements. CT and MRI findings showed global atrophy, thinning of the corpus callosum and delayed myelination. There was no facial dysmorphism but other features were consistent with those described in patients with LoF *SMC1A* variants (192,193). Two patients had variants involving *CDKL5,* the first of which was a splice variant located within the kinase domain, detected in a female patient (EIEE2) with neonatal-onset generalised tonic-clonic seizures (GTCS), evolving into daily myoclonic jerks. The seizures were refractory to treatment, though improvement was noted on the ketogenic diet, which was abandoned after three months, due to its expense. No parental DNA was available for testing, but the variant was classified as pathogenic based on the predicted LoF (canonical splice), its position within the critical domain of the gene and protein, as well as strong clinical correlation. The second *CDKL5* variant was a whole gene deletion identified on DNA microarray in a female with GTCS evolving into myoclonic jerks (EE57). The age of seizure onset was uncertain, as the child was seen at the clinic for the first time at 23 months of age. The clinical features in both girls were typical of CDKL5 deficiency and included myoclonic jerks, hypotonia, feeding problems (gastrostomy

fed), cortical visual impairment and profound DD (194,195). Seizures in both patients were refractory to treatment. In March 2022, the FDA approved Ganaxolone for the treatment of seizures associated with CDKL5 deficiency disorder (CDD) in patients 2 years of age and older (196). This should present a new therapeutic option to these patients, though the drug is not as yet available in SA.

Sixteen variants in 12 patients were classified as VUS, as insufficient evidence was available to determine disease association according to the ACMG criteria (1). Four of these may be reclassified as P/LP should DNA from both parents become available for segregation studies to show *de novo* origin (Suppl. Table 1, GIM paper). One patient (EE141) had a novel 3Mb deletion, involving the non-morbid *NRG3* gene, which may be of significance, if only by virtue of its size [though the gene itself is not under functional constraint (pLoF = 0.26), arguing against its pathogenicity]. One patient (EE47) tested compound heterozygous (in *trans*) for variants in *UNC80,* as well as a VUS in *COBLL1. UNC80* is a known morbid gene associated with Infantile Hypotonia, with Psychomotor Retardation And Characteristic Facies 2 (IHPRF2), a severe AR neurodevelopmental disorder with onset at birth or in early infancy, and epilepsy in some patients (197). The patient, however, was only mildly affected exhibiting few of the features described in IHPRF2 [strabismus, slow acquisition of skills, abnormal EEG and (now controlled) epilepsy]. The *UNC80* variants previously associated with the severe IHPRF2 phenotype were all truncations, whereas those found in our patient were missense. Therefore, considering the limited current knowledge, perhaps the possibility of a milder phenotype linked to missense *UNC80* variants should not be discounted, possibly supported by the *in-silico* predictors of impact on protein function (SIFT, PolyPhen, CADD) and absence from population frequency datasets (15). The same patient also had a *de novo* splice variant in *COBLL1*, a brain-expressed gene of unknown function. Interestingly, *COBLL1* ranked as the third most prominent gene for DEE analysis (after *SCN1A* and *NEXMIF*) in the Epi25 study of ultra-rare genetic variation in 17 606 individuals, though it did not meet genome-wide significance (38). However, the significance of this variant in absence of additional evidence is unknown. Another interesting candidate was a heterozygous, *de novo* single nucleotide substitution in the *GLUL* start codon, in a patient with refractory epilepsy, profound GDD, dysmorphology and structural brain abnormalities (Suppl. Table 1). *GLUL* is a known gene implicated in glutamine deficiency but a role in epileptogenesis has been suggested (198), owing to some evidence linking *GLUL* deficiency with neonatal-onset, severe epileptic encephalopathy (199,200). In an effort to solve some of the candidates detected with ES (Supp Table 5, GIM paper), the GeneMatcher platform was used to establish possible connections with other investigators who may be working on the same genes/variants, in similarly affected patients (16). In this way, a connection was established with a group working on the *GLUL* gene (manuscript in preparation).

The absence of *KCNQ2* variants from the cohort was noted with surprise, as it is the most frequent genetic cause of neonatal seizures according to international reports. As indicated in the GIM paper, these patients may be un/misdiagnosed in Africa on the presumption of hypoxia or infection as the most common cause for neonatal seizures. This is an important issue, as the diagnosis has important therapeutic implications. The *KCNQ2* seizure semiology is relatively specific and should lead to the clinical suspicion and targeted treatment with ion channel blockers. In a tertiary hospital setting with neonatology and neurology services, this may take place if recognized, but only once the seizure pattern is established, which can be some weeks into the admission. This conservative

approach is justified by the concern that with no access to genetic confirmation, seizures may worsen on incorrect treatment. This highlights not only the need for access to early testing but the importance of multidisciplinary consultation and creating awareness among the paediatricians and neonatologists in SA.

The high CNV detection rate (13/78, 17%) was an unexpected and important finding of the study, especially since only 78 patients were CMA-tested (Suppl. Table 1, GIM paper). CMA became available to SA patients in the state healthcare sector mid-way through the study (2019) and some of these patients were clinically tested and diagnosed post-recruitment. Whilst these CNVs may be seen to inflate the CNV detection rate in the study (which may be even higher, if all children were CMA-tested), they were not excluded from subsequent statistical analyses, as the goal was to assess a real-life patient population in a paediatric epilepsy clinic in SA. In addition to raising awareness, diagnosing these patients through research stressed the importance of periodic re-assessment of undiagnosed patients and an ongoing search for new diagnostic tools and interventions. In some cases, the genetic diagnosis enabled more holistic care involving additional teams, including clinical genetics and neurodevelopmental medicine. As noted in the GIM paper, genetic counselling and cascade testing could be offered to the families of the three patients with maternally inherited CNVs (22q11.21 gain, 16p13.11 loss and 16p13.11 gain). Moreover, a genetically confirmed diagnosis often carries practical implications for children with ID/DD phenotypes (and their parents or carers), as it may facilitate applications for placement in special schools and access to auxiliary services.

Three patients in the study group (EE47, EE62 and EE107) each had more than one variant of possible or likely clinical significance. A genetic “double-hit” from different epilepsy loci is not especially unusual and may have a combined effect on the phenotype (201,202). Interpreting the significance of the variants in such cases (and in general), is greatly aided by demonstrating *de novo* occurrence and absence or low frequencies in datasets of normal variation in the relevant population. Both these aspects present a practical challenge in the African context as 1) DNA from both parents may not be available and 2) there is limited access to variant frequencies in SSA populations. Therefore, taking patient EE62 as an example, in the absence of paternal DNA, *de novo* occurrence and likely pathogenicity of the *KCNA2* could not be proven, creating a genetic counselling challenge. In such cases, careful clinical correlation is essential, with an attempt to access paternal DNA. More genomic sequencing and greater availability of allele frequencies in African populations is needed to aid the interpretation of such variants in individuals of African, especially SSA descent.

Accurate phenotyping and population of the REDCap database (Appendix 3) was a major undertaking of this study, quite underestimated at the onset, in terms of the detail and time required. Collecting clinical information and then later, actioning the genetic results required Multidisciplinary Team (MDT) involvement, which added an unexpected, highly valuable educational component to the paediatric community. It also highlighted the importance of meticulous patient and parent interview, careful noting of the seizure semiology and evolution, ages of onset and the subtle signs and symptoms, for possible later correlation with the putative genotype (e.g., prolonged febrile seizures starting at <6 months in DS (181), stereotypical hand movements with *STXBP1* epilepsy(112), early eyelid stereotypy in DS (188), etc.).

The experience of this study emphasised the importance of multidisciplinary input in determining the true significance of the detected variant in terms of the diagnosis, possible impact on treatment and any prognostic value that the result may carry for the patient and the family. In a disorder with a wide genetic and phenotypic overlap, the detected variant may inform the diagnosis, or the clinical presentation may aid variant interpretation. Ideally, a MDT of medical specialists and scientists should be involved in establishing the molecular and clinical diagnosis and most appropriate treatment. The family should then be counselled about the genetic finding and its implications. This type of specialist team input is currently available in only a few tertiary healthcare settings in SA and introduction of genetic testing for epilepsy will necessitate augmentation of both the existing laboratory and as well as the clinical services. However, such obstacles can be overcome with sufficient political will, which can be rallied for an important and currently lacking service.

5.2. Challenges

In the SA day-to-day practice, recognition of possible signs of a genetic epilepsy is complicated by the layering effects of TB, HIV, parasitic and febrile illness, perinatal insult, as well as poor nutrition and other complications of the socio-economic circumstance. Access to care, shortage of specialist skills (both clinical and laboratory), the stigma and cultural beliefs surrounding epilepsy in many communities, as well as the many socio-economic challenges experienced by the African patients and families were addressed in the FIN paper incorporated into Chapter 2 (Literature Review) of this dissertation (203).

The challenges encountered during the research study are also likely to impact the service. For example, recruitment of genetically naïve patients in a real-time clinical setting, resulted in inclusion of patients with yet unidentified, acquired causes or other diagnoses (detailed in the GIM paper), which ultimately lowered the variant pick-up rate. In local clinical practice, future diagnostic test requests will come from similar or less specialised backgrounds, hence, strict gate-keeping will be required to avoid inappropriate testing and unnecessary expenditure. This goes hand-in-hand with creating awareness, facilitating consultation and education, as patients may be referred by non-neurologists. Effective gate-keeping can only be rendered by specialists and requires establishing administrative processes and lines of communication between the clinicians, the laboratory and the hospital management, adding an additional level of complexity. A major contributor to the level of therapeutic success is the ability to limit the time elapsed between the seizure onset and embarking on the correct diagnostic and therapeutic course. The Think-Genetics decision tree described in the GIM paper, was proposed to reduce such delays and simplify decision-making at patient entry in resource constrained settings, for an early, correct intervention.

Variant interpretation in DEEs relies heavily on the ability to demonstrate *de novo* occurrence (19,204). The difficulties frequently encountered in accessing DNA from both biological parents in the African setting have already been addressed in the discussion of the FIN paper (203). The clinical significance of several VUS identified in this study may be resolved if DNA from both parents were available for segregation analyses. In such cases, accurate phenotyping and detailed noting of the clinical features becomes even more important, emphasising the relevance of defining the phenotypic spectra and genotype-phenotype correlations. To our knowledge, no detailed genotype-phenotype studies have been done on African individuals with epilepsy,

presenting a research topic which may reveal useful additional insights. There is also limited available data on genetic variation and allele frequencies more broadly in African populations, especially in SSA. It is hoped that the data already generated through initiatives such as the H3Africa and the AGV projects (34,35) will become more widely accessible, as the present silo-ing of this data limits its utility for clinical variant interpretation.

Genomic research on a meaningful scale requires large capital investment and a skilled workforce. Research in African populations is often limited to the level of sample collection, with the actual research conducted in well-funded laboratories of HICs. The recent efforts to fill the gaps in genomic knowledge and capacity in Africa have been facilitated by international funding agencies (34). However, translation of NGS-based research into routine local clinical testing protocols is hampered by the persistently high cost. The promise of a drop in reagent costs has not materialised tangibly in SA. Suppliers base price negotiations on the projected throughput, which is low due to the high cost of reagents. Indirectly, this also affects the test turn-around-time (TAT), as sample batching is essential for cost-efficient utilisation of reagents. However, few patients are tested owing to the expense, thus collecting a “batch” takes longer and prolongs the test TAT. Appropriately skilled molecular geneticists and bioinformaticists, even if available, are viewed as expensive and few posts are available. Bioinformaticists in South Africa occupy the research realm almost exclusively, and the professional category of a Clinical Bioinformaticist does not yet exist in Africa.

Therefore, setting-up a NGS-based diagnostic testing service on a meaningful scale may be viewed as non-feasible, with local users and health administrators turning to international service providers, for cheaper (though still expensive) and quicker testing, with no need for capital investment, staff recruitment or the expense of accreditation. The risk accompanying this arguably short-sighted solution was recently demonstrated by the local experience with Invitae laboratories (USA), whose abrupt withdrawal of institutional support in September 2022 drastically limited or removed the availability of diagnostic NGS panels for patients in the SA state sector. The positive side-effect of this experience was that the resulting gap in service highlighted the clear benefits and clinical utility of NGS for epilepsy (and other disorders), which could be used to motivate for the resources to offer NGS-based testing locally.

5.3. Strengths and Limitations

The unbiased, real-time recruitment and broad inclusion criteria purposely reflected the local patient referral and triage systems, resulting in inclusion of patients diagnosed with DEE of other, not yet established aetiologies or acquired causes (details within the GIM paper). The true overall P/LP variant pick-up rate was therefore likely to be higher than the 17% reported in the GIM paper. It was decided not to exclude these patients from the statistical analyses, as the clinical demographic of the cohort reflected that of the busy tertiary epilepsy referral centre. Children from across the spectrum of SA primary health care presented either directly to the hospital, or via specialist referrals for (refractory) seizures as the main concern. Recognition of a possible genetic epilepsy from an array of seizures of various, mostly acquired aetiologies is a challenge, as awareness of genetic causes is limited in absence of genetic testing. Therefore, the descriptive statistics and logistic regression modelling were performed on all patients who fulfilled the recruitment criteria, in an effort to identify clinical features, which may stand out at patient entry, as possible indicators of a genetic aetiology.

The REDCap clinical database used for data collection was populated retrospectively, using patient folder entries previously made by various healthcare staff, in different clinical settings. For the sake of consistency, information was captured by one clinical team, who met frequently to discuss cases. Often, available information was incomplete or poorly recorded (e.g., seizure onset, type and evolution, ASMs trialled over time, etc.) and had to be preferentially excluded from the database and subsequent statistical analysis. Multivariate analysis could only be undertaken using variables for which data was available for most patients, resulting in only a subset of analysable variables. This, and the relatively small sample size of 234 children were a limiting factor to the statistical outputs, and a possible source of bias. Nonetheless, the REDCap database proved to be an effective tool for data collection, allowing for some statistical analyses and inferences. A few clinical factors appeared to indicate association with the presence/absence of a SNV or CNV, though statistical significance at a threshold of p-value <0.05 could not always be achieved.

The variance and discrepancies in the clinical information recorded in the patients' folders were not surprising, as the clinical assessments were made over time by a wide range of clinicians, in children at different brain-age time frames. It was useful and interesting to note the inter-observer variation from parental reports and clinicians' mis/interpretation of features and events. The records told a story of how, for each child, the seizure semiology evolved, in some cases changing the epilepsy syndrome label over time. It highlighted the need for precision therapy based on targeted diagnostic tools such as a genetic test, which can potentially lead to a diagnosis with immediate implications for treatment and prognosis, removing the need for further investigations. The records also highlighted, especially among the children with the microdeletion/duplication syndromes, how certain features may go under-reported by parents and be missed altogether even by the clinicians. The onset (or worsening) of seizures is often the main reason parents seek help, but the preceding issues may only become apparent on careful questioning.

The study outcomes suggest that it may be possible, with a larger and more complete dataset, to build and validate a more powerful predictive algorithm to ensure early genetic diagnosis and appropriate treatment, as well as an economically viable use of clinical and laboratory resources in SSA and the LMICs more broadly. Importantly, the decision tree described in the GIM paper was based on the statistical and genetic outcomes of this research and does not encompass all possible clinical scenarios. It was not designed to replace specialist clinical insights or imply that genetic testing is inappropriate for patients whose clinical features or age at seizure onset vary from those incorporated into the decision tree. Its purpose was to alert the entry-level clinician, specifically in the resource limited settings, to a possible genetic aetiology in the early-onset cases, where an early diagnosis and intervention is often critical.

5.4. Future directions

The directions and opportunities for future epilepsy research in Africa are almost limitless, owing to the great burden of epilepsy on the continent and limited research conducted to date. In addition to the need for training and educational initiatives described under Conclusions and Future Directions in the FIN paper, there is great scope for genetic epilepsy research on the wholly under-investigated African patient population.

In terms of future exploration flowing directly from this study, whole exome and/or long-read genome sequencing is the next logical step in investigating the remaining patients and will be incorporated into future studies. Another important project will involve a group of children in the RCWMCH service, who were initially diagnosed with epileptic spasms and later found to have TSC. *TSC1/2* genes were not included in the DEE panel used in this study. The children were to be investigated for variants in the mTOR regulatory genes, along with other TSC patients in the RCWMCH service, as a dedicated objective. It was however decided that CNV analysis in the existing cohort should be prioritised. Preliminary work on the TSC group has been conducted, however a more in-depth genetic study of the malformations of cortical development is in the planning stages. The TSC genes have been included in the DEE panel design for local diagnostic use.

African genotypes are significantly underrepresented in the ongoing large-scale GWAS, such as that published by the ILAE Consortium on Complex Epilepsies (103), in search of loci associated with the common epilepsy phenotypes (generalised and focal), which remain largely undetermined. The reasons for this underrepresentation are varied and relate partly to the practical and financial aspects of participant recruitment and sample transport within the logistically challenged setting, and partly to issues concerning data and genomic material ownership. These obstacles, however, can and should be overcome, as many funding agencies recognise the importance of diversity in genomic research, which will benefit all.

The pharmacogenomic aspect of epilepsy is an exciting and growing field. Response to ASMs is highly variable and seizure control often involves a lengthy period of trial and error. The rate of drug resistance in epilepsy is high, its basis complex and not well elucidated. In recent years, over 120 genes involved in ASM metabolism, transport and target pathways were revealed as important modulating factors of drug response (205). Whilst more research and clinical testing is required to assess pharmacogenomics-directed care, investigators suggest that in not-too-distant future, pharmacogenomic loci may become incorporated into panel (or virtual panel) testing, potentially revealing not only the underlying aetiology of the epilepsy, but also information on to ASM metabolism in the individual, leading to personalized treatment (206). Here, again, sufficient pharmacogenomic data must be generated and analysed to identify informative, population-relevant loci. The genetic drivers of ASM metabolism in individuals of SSA descent are currently largely unknown. In a continent with the highest burden of epilepsy worldwide, the pharmacogenomics of ASMs presents an exciting opportunity for meaningful research.

Translation of the research findings and developing a genetic diagnostic service for the DEEs was the main aim of the study. To this end, internal validation of a DEE gene panel is currently underway in the diagnostic Genetics laboratory of the South African National Health Laboratory Service (NHLS) in Cape Town. The utility of the Think-Genetics decision tree described in the GIM paper and the diagnostic yield of the local panel testing will be assessed by measuring patient-relevant outcomes.

5.5. Conclusions

This dissertation describes the results of the first genetic epilepsy research study conducted in SSA. As such, this work makes an important contribution to the knowledge of the genetic architecture of paediatric epilepsies

in patients on the African continent. Future epilepsy research in Africa with its great genetic diversity will, no doubt, add new and useful insights. However, translation into service was an important, short-term aim of this study and the immediate challenge lay in proposing diagnostic protocols, which could be adopted and sustained within the financially and logistically constrained local healthcare system. The genetic results confirm the unquestionable value of genetic testing in DEE, where identification of the causative variant may bring an end to the diagnostic odyssey, enable targeted treatment and facilitate genetic counselling. The study outcomes also emphasised the importance of raising awareness of genetic aetiologies in epilepsy, ongoing education and MDT involvement. Teams comprising of neurologists, geneticists, counsellors and molecular scientists are available in only a few tertiary healthcare centres in SA, however access to support and consultation could be facilitated by using electronic means e.g., online meeting tools. This does not, however, negate the obvious need for significant augmentation of the existing genetic laboratory and clinical resources in SSA.

The infrastructure required to conduct this research is another, important output of the study. The ethical and institutional approvals, a well-designed and functional REDCap database, established patient recruitment and consenting process and lines of MDT communication, provide the groundwork to be used and built upon with future research into genetic epilepsies in African patients.

5.6 Research outputs

In addition to the publications included in this thesis, the study findings were presented at the following conferences and seminars:

Local:

Oral: *Genetics of Developmental and Epileptic Encephalopathies in South African Children – Relevance to Precision Medicine.* UCT Department of Paediatrics & Child Health Research Day (October 2022), Cape Town, SA.

Oral: *Genetics of Developmental and Epileptic Encephalopathies in South African Children – Relevance to Precision Medicine.* NHLS ECHO (Extension for Community Healthcare Outcomes) project (June 2022) (online).

Oral (invited speaker): *Epilepsy Genetics – What is All the Fuss About?* 45th Annual UCT Paediatric Refresher Course (February 2022), Cape Town, SA.

Oral (invited speaker): *Epilepsy genetics: what should we be doing in SA?* Paediatric Neurology and Neurodevelopment Association of Southern Africa (PANDA) meeting (October 2018), Cape Town, SA.

Oral: *Dravet Syndrome: Genetic Causes and Diagnosis in A Cohort of Children from Red Cross War Memorial Children's Hospital*; UCT Department of Paediatrics & Child Health Research Day (October 2017), Cape Town, SA.

Oral: *Dravet Syndrome: Genetic Causes and Diagnosis in A Cohort of Children from Red Cross War Memorial Children's Hospital*; UCT Department Pathology Research Day (November 2017), Cape Town, SA.

Oral: *Delineation of the Genetic Causes of Epileptic Encephalopathies in South African Paediatric Patients.* South African Society of Human Geneticists (SASHG) Congress (August 2017), Durban SA.

International:

Oral (invited speaker): *Advances in Epilepsy Genetics: Derived Concepts for Clinical Care of Patients,* 4th Africa Epilepsy Congress (August 2019), Entebbe, Uganda.

Poster: *Delineation of the Genetic Causes of Epileptic Encephalopathies in South African Paediatric Patients.* 32nd International Epilepsy Congress (September 2017), Barcelona, Spain.

5.7. References

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6. SUPPLEMENTAL INFORMATION

The content of this chapter is similar to that in the Supplemental Information of the GIM paper, including the naming and numbering of the Supplementary Tables and Figures referred to in the article.

6.1. Supplementary Tables

Suppl. Table 1. **Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
CNV284/M	6 years	GTCS with focal onset: twitching of left face and side, becoming generalised, 1-3/month, often with intercurrent infection	**PB, LTG, RIS; refractory**	severe GDD, wheelchair-bound, non-verbal, stereotypies	profound GDD/ID	stereotypies	upper airway obstruction (tracheostomy), large ears, recurrent lower respiratory tract infections, high arched palate, hearing impairment, cortical blindness, coarse facies, facial asymmetry, bilateral postaxial polydactyly, bradydactyly, bilateral inguinal hernias	Generalised slowing for age	CT: normal	12p13.33p11.1 mosaic gain [0.02%]	Pallister Killian Syndrome	Diagnostic closure and genetic counselling. MDT support for ongoing management.
DS1/M	2 months	febrile, prolonged focal with bilateral synchrony 1-3/month; later focal, GTCS, tonic, myoclonic 1-3/month; SE (ICU with ventilation)	PB, CBZ, LTG, **VPA, LEV**, KD; **refractory**	normal	mild DD (DQ 50), language delay, potty training at 4 yoa, neuroregression from 1 yoa due to treatment-resistant seizures, recurrent hospital admissions	loss of independent walking	none	normal at presentation	CT, MRI: normal	*SCN1A:* c.5314G>A,(p.Ala1772Thr), recurrent (ClinVar ID: 68570; PMID: 30321769; 17347258; rs121917980), P	Dravet syndrome (score 10)	Diagnosis and implications for treatment: initially clinically recognised as DS but the genetic confirmation assisted the mother plan placement and care.
DS10/M	8 months	febrile, focal, GTCS, myoclonic 1 -3/month; later GTCS <1/month	PB, **VPA**, PB, LEV, CZP, CBZ, **TPM**, KD; **refractory**	normal	DD (DQ 66), aggressive behaviour, ASD	slowness of mobility	none	performed 11 years old: slow in frontal regions and generalised spikes	MRI: normal	*SCN1A:* deletion of exons 5-8, P, LoF	Dravet syndrome (score 7)	Initially clinically recognised as DS but an earlier genetic confirmation would have given family closure. Referred from the private sector where managed for immunodeficiency related to the recurrent febrile seizures.
DS11/F	3 months	focal, generalised tonic, myotonic, GTCS <1/month, later increasing to 5-9/day, triggered by bathing and fever; SE (ICU with ventilation)	PB, CZP, **VPA**, TPM, LTG, PHT, **STP, CLB, KD; refractory**	normal	language delay, learning difficulties (DQ99), behavioural issues, ASD	crouched gait, scoliosis, action tremor, slow and hesitant movements	none	generalised slowing and interictal discharges with left centrotemp predominance and frontal polyspike	MRI: normal	*SCN1A:* c.2552G>C,(p.Arg851Pro), recurrent (ClinVar ID: 98593; PMID: 26096185; 20110217; 30321769; rs121918785), de novo, P, LoF	Dravet syndrome (score 11)	Clinically recognised as DS but the genetic diagnosis enabled marked rationalisation of polytherapy and motivation for access to stiripentol and medical insurance cover. The genetic result significantly influenced the parents' perceptions, with all family plans adjusted around the confirmed diagnosis.
DS12/F	6 months	febrile, GTCS, a few/week; subsequently generalised atonic 5-9/day, triggered by heat and excitement; SE (PICU admission)	**VPA**, PB, CLB, **CBZ**, LTG, **TPM**, LEV**; refractory** (passed away at 16yoa, ?SUDEP)	normal	neuroregression (special school)	No	none	normal (one EEG on initial presentation)	CT: normal	*SCN1A:* c.4016T>G,(p.Val1339Gly), de novo (PMID: 30321769[b]), P	Dravet syndrome (score 9). SUDEP	Clinically recognised as DS but genetic confirmation and counselling about the risk of SUDEP provided closure to the family.
DS14/F	6 months	febrile, generalised clonic, GTC, myoclonic a few/week, frequent SE requiring ICU support	**CZP**, **STP**, CBD, **VPA**, Tegretol, LEV, TPM, LTG, Acetazolamide, CLB, KD; **refractory**	normal	severe DD (DQ 40) , autism, hyperactive, neuroregression due to severe and recurrent, prolonged seizures in infancy	No	none	background evolution from normal to slowing at the right temporal region	MRI: normal	*SCN1A:* c.5236G>T,(p.Gly1746Trp), novel (PMID: 30321769)[b], LP, LoF (PMID: 35037686)	Dravet syndrome (score 12)	Initially clinically recognised as DS but the genetic diagnosis permitted targeted and more appropriate care.

Suppl. Table 1 cont. **Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
DS19/F	4 months	febrile, focal <5/day; subsequently focal, GTS, myoclonic <5/day	**VPA, LTG**, CZP, CBZ, TPM**; refractory**	normal	moderate DD and autistic features	No	very drowsy/encephalopathic, chronic tremor	generalised slowing	MRI: global atrophy, mild generalized cerebral atrophy	*SCN1A:* c.4352_4356del,(p.Tyr1451CysfsTer22), PMID: 30321769[b], P, LoF	Dravet syndrome (score 9)	Clinical reassessment and a revised diagnosis with implications for treatment. Current ASMs sub-optimal and likely require adjustment. However, difficulty with follow-up, as patient living in a distant town and only seen once.
DS2/F	4 months	prolonged febrile, focal, generalised clonic <1/month, later increasing to 10 - 19/day, durinal and nocturnal; SE (PB loaded, intubated and ventilated)	**VP, CLB, LEV, STP; refractory**	normal	GDD with moderate ID	behavioural issues	none	initially normal but multifocal and generalised abnormalities with established epilepsy.	MRI: normal	*SCN1A:* c.3007delA,(p.Ile1003LeufsTer10), novel (PMID: 30321769)[b], P, LoF	Dravet syndrome (score 7)	Initially clinically recognised as DS but the genetic confirmation was of great importance to this family, helping to answer questions, provide care directions and expectations, as well as redirect compliance.
DS22/M	3 months	febrile, GTCS 1-3/month; later focal a few times/week; SE (PB loaded)	**VPA, PB, LTG,** CLB, LEV, TPM, KD; **refractory**	delayed smile	language delay; neuroregression from 2 yoa due to multiple SE and frequent breakthrough seizures	wide based unsteady gait	dysmorphism (epicanthic folds, flat nasal bridge fish-like mouth, up-turned upper lip, large ears), ADHD	generalised slowing	CT, MRI: global atrophy	*SCN1A:* c.1129C>T,(p.Arg377Ter), recurrent (ClinVar ID: 189963; PMID: 30321769; 27465585; rs794726799), P, LoF	Dravet syndrome (score 9)	Initially clinically recognised as DS. Lost to follow up but track down and booked in to rationalise ASMs (get off LM), and possibly motivate for access stiripentol.
DS4/M	5 months	focal 1-3/month, later focal, GTCS, myotonic, absence <5/day, triggered by water, sunlight, excitement; SE (valium and PB)	PB, VPA, CBZ, **TPM**, LEV, **ESM**, **CZP**; **refractory**	normal	GDD with learning difficulties (DQ 85); VMI problems: BEERY = 3 years; draw a man = 3years, attention deficit	myoclonic jerks	none	3Hz generalised epileptiform activity with a right frontal take off. Clinical correlation head nods, lasting 3-30 seconds with occasional left eye	MRI: mild left hyperintensity in the left subcortical regions	*SCN1A:* c.664C>T,(p.Arg222Ter), recurrent (ClinVar ID: 12889; PMID: 23195492; 11359211; 29100083; 30321769; rs121918624), P, LoF	Dravet syndrome (score 15)	Diagnosis and implications for treatment: referred for a "focal seizure disorder", dramatic improvement on stopping CBZ, resolving myoclonus for several years.
EE10/M	2 months	mostly on waking; subsequently GTS, GTCS, absence 10-19/day, triggered by fever; SE (PB	**VPA, CLB, TPM, LEV**, PB, LTG, CBZ, PN, KD; **refractory**	mild right hemiplegia initially reported	autistic features, drooling, walks unaided but needs assistance with dressing and grooming	hand stereotypies, self-stimulation behaviour	none	generalised slowing (more event on the right)	CT, MRI: normal	*SCN1A:* c.4444-1C>T, recurrent (ClinVar ID: 530456; PMID: 18930999; 17347258; rs1553521567), P, LoF	Dravet Syndrome (score 10)	Clinical reassessment and a revised diagnosis with implications for treatment (previously worsened on CBZ and LTG).
EE103/M	24 months	generalised myoclonic (jerks) and GTCS 5-9/day, later increasing to 10-9/day, generalised myoclonic seizures in sleep	VP, LTG; **seizure-free** and off ASMs.	moderate ID, attending special school	lost to follow up	No	mood swings, behavioural problems	Generalised spike and wave	CT: normal	22q11.21 (gain), present in mildly affected mother	22q11 microduplication syndrome	Stopped attending the epilepsy clinic but encountered by the genetics team during an outreach trip to the special school. Counselling about the genetic diagnosis and cascade testing extremely valuable to the family with multiple apparently affected individuals. Emphasised the importance of MDT support.
EE104/F	10 months	febrile <1/month, later febrile, GTS, GTCS 1-3/month	**PB; controlled**	severe cognitive delay, evolving CP	severe DD (DQ 40)	No	soft dysmorphism: hypertelorism, low nasal bridge; cortical visual impairment; ASD secundum x2.	not recorded	CT, MRI: thinning corpus collosum, evidence of HIE, queried lysosomal storage disorder	4p16.3 (loss)	Wolf Hirschhorn syndrome	Diagnostic closure of a well delineated condition, and genetic counselling. MDT support includes a dedicated clinician with an interest in this condition, who coordinates care for all affected children.
EE107/M	3 months	febrile, GTCS, myoclonic jerks <1/month; later focal, GTCS 5-9/day	PB, VPA (mother stopped ASMs in favour of herbal/traditional medicine); **refractory**	HIE, CP, dystonia, poor head control, likely cognitive delay	profund DD (DQ 40); loss of social interaction and developmental progression, hyperactive	No	CP, dystonia, cortical visual impairment, duplex kidney	No epileptiform activity detected at presentation	CT, MRI: global atrophy, thinning corpus callosum, HIE and mod large perivascular spaces	5q35.2 (loss); *SCN3A* : c.3838G>A (p. Val1280Ile), recurrent VUS (rs751582800), paternal DNA unavailable for segregation.	Sotos syndrome	Diagnostic closure of a well delineated condition, and genetic counselling. MDT approach and targeted support for ongoing management of a patient with limited resources.
EE11/F	7 months	GTCS <5/day; later focal 5-9/day; SE	**VPA, LEV**, PN, Folinic Acid**; refractory**	normal	neuroregression post seizure clusters: motor, verbal and cognitive, hypersensitive to noise and tactile stimulation	No	none	normal at presentation	CT, MRI: normal	*PCDH19:* c.2512C>T,(p.Gln838Ter) , novel (PMID: 30321769)[b], de novo, P, LoF	Epilepsy and Mental Retardation Limited to Females (EFMR)	Diagnostic closure, genetic counselling and possible cascade testing.

Suppl. Table 1 cont. **Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
EE113/M	12 weeks	spams <5/day for 12 weeks; seizure-free on treatment	ACTH, VPA; **seizure-free**	mildly delayed	decline in head circumference and GDD	No	none	chaotic modified hypsarrhythmia; evolved on treatment to generalised slowing with isolated bilateral spike	MRI: Loss of peritrigonal white matter	*NARS:* c.1600C>T, (pArg534Ter), recurrent (ClinVar ID: 982711; PMID: 32738225), de novo, P, LoF	DEE / *NARS* gene encephalopathy	Diagnostic closure and genetic counselling. Important for the family to know that the spasms were not related to birth anoxia.
EE12/F	4 months	febrile <1/month, later 10-19/day; SE	**VPA, PB (weaning),** LTG**, CLB,** LEV**, TPM (low dose), STP, KD** (early on)**; refractory**	normal	profound DD, loss of speech, poor social interaction, drooling	crouched gait, repetitive hand movements	precocious puberty, hyperactivity, autism, glaucoma	generalised slowing	CT, MRI: HIE	*SCN1A:* c.2803C>T,(p.Arg935Cys), recurrent (ClinVar ID: 68604), P, LoF	Dravet Syndrome (score 9)	Now on stiripentol and weaning PB. Clinically recognised as DS but more focused discussions and counselling on treatment interventions after genetic diagnosis.
EE125/F	4 years	complex febrile, GTCS a few/week	LTG, LEV, CZP, CLB, **VPA, CBZ, PB**; **refractory**	autism and ID evident from infancy	profound global DD (DQ 40)	No	soft dysmorphism: flat nasal bridge, hypertelorism, small ears, full lips and bulbous nose; scoliosis, aggressive and hyperactive.	parieto-occipital sharp waves evolving to left parietal discharges.	CT, MRI: normal	*SCN2A:* c.4551+1G>A, recurrent (rs527688117), P, LoF	DEE - good electroclinical correlation for *SCN2A*	Diagnosis and implications for treatment. Earlier knowledge of genetics would have supported a targeted approach to treatment i.e. reassessment of CBZ for the LoF *SCN2A* variant.
EE126/M	5 months	hemiclonic <5/day; later hemiclonic, focal during sleep <5/day, a short daily myoclonus; SE	**PB, VPA**, CLB**; controlled**	normal	profound ID, hypotonia, loss of language and mobility	hand stereotypy, ataxia - broad based gait - eventually lost ambulation.	pulmonary TB	generalised slowing	MRI: normal	*STXBP1:* c.1651C>T(p.Arg551Cys), recurrent (ClinVar ID: 207440: PMID: 32112430; 23409955; 26514728; rs796053373), P	DEE / *STXBP1* encephalopathy	Diagnosis and implications for treatment.
EE127/M	3.5 months	FMS, episodes of rapid horizontal eye movements, staring, eyelid fluttering, tonic flexion and elevation of upper limbs with associated downward eye deviation 20-100/day; later ES, FMS, myoclonic and atypical absence-like seizures	various (no detail available), some imrovement with VNS but still poor seizure control; **refractory**	normal	severe GDD, visually impaired, non-verbal, increased tone on the left, walks with help	No	none	posterior epileptiform abnormalities (bi-occipital and temporal spikes).	MRI: normal in infancy but generalised white matter atrophy and thin corpus callosum at 10 years.	*KCNT1:* c.1496A>G,(p.His499Arg), de novo, recurrent (ClinVar ID: 449802; rs1554774362), P	DEE - probable Early Infantile Migrating Focal Seizures /*KCNT1* encephalopathy	The delayed genetic diagnosis restricted early and targeted intervention, e.g., quinidine trial.
EE20/M	15 months	GTS, a few/week (body "pulled stiff"), subsequently GTCS, brief episodes of eye-blinking	**VPA, LTG; refractory**	concerns about language delay	moderate DD (single words, behavioural problems, attends LSEN school)	crouch gait, motor stereotypies	none	generalised slowing	MRI: normal	*CHD2:* c.2095C>T,(p.Arg699Trp), recurrent (ClinVar 429658; rs1131691515), LP	DEE/*CHD2* gene encephalopathy	Diagnostic closure and genetic counselling. Better clinical prognostic expectations related to gene encephalopathy.
EE21/F	9 months	febrile, focal 1-3/month, later focal, GTCS, GTS <1/month	**VPA, CBZ, LTG; refractory**	normal	ID: attends LSEN school	No	macrocephaly, cafe au lait spots (does not meet NF criteria)	normal	MRI: normal	*SCN1A:* c.1520A>T,(p.Lys507Ile), novel, VUS but LP if de novo (paternal DNA unavailable for segregation)	Dravet Syndrome (score: 7)	Diagnosis with implications for treatment. Requires follow up on the use of LTG.
EE22/F	2 weeks	GTCS a few/week; later GTS, GTCS; SE (PB loaded)	**PB, VPA**, VGT**; seizure-free**	normal	DD (non-specific), autism	hand stereotypy	dysmorphism: unilateral cleft lip, tapering of fingers, eyelid haemangiomas, overriding 2nd and 3rd toe on the right; visual impairment progressive deterioration.	normal	CT, MRI: normal	*STXBP1:* c.1099C>T,(p.Arg367Ter), recurrent (ClinVar ID: 207429: PMID: 31344879; 28944233; rs796053366), de novo, P, LoF	DEE/*STXBP1* gene encephalopathy	A well defined gene encephalopathy. Implications for treatment: controlled on current agents LEV is an option if required
EE35/M	8 years	focal <5/day, subsequently focal, GTCS a few/week	**VPA, CBZ; good response to CBZ** but poor initial adherence (low CBZ levels)	normal	DD and neuroregression: episodes of confusion, headaches, aimless wandering, combative outbursts, limited verbal communication (ASD spectrum); stopped attending school	unsteady gait	none	generalised slowing	CT, MRI: normal	*SCN2A:* c.5836A>G,(p.Lys1946Glu), VUS/LP (LP if de novo but paternal DNA unavailable for segregation).	DEE/*SCN2A* gene encephalopathy (likely)	Markedly improved seizure control upon adherence to CBZ. Earlier testing would have permitted earlier, targeted treatment and prevented extensive investigations (including autoimmune work up).

Suppl. Table 1 cont. **Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
EE42/F	6 months	myoclonic shoulder jerks 5-10/day, often on waking; later fever-triggered episodes of staring with altered consciousness	**VPA; seizure-free**	floppy, poor hand use, not reaching to grasp, unstable sitting, rounded back, unable to roll prone to supine.	severe global delay with autistic features	hand stereotypies, jerky shoulder movement, dystonic movements of the hands	Haemangioma on forehead, epicanthic folds, flat nasal bridge, short philtrum.	frequent bilateral spike and wave discharges and polyspike paroxysms	thinning of the corpus callosum splenium and abnormal peritrigonal white matter	*FOXG1* deletion on CMA	*FOXG1* syndrome	Diagnostic closure, genetic counselling and possible cascade testing.
EE47/F	2 months	focal <1/month; later focal, generalised myoclonic once/week	LEV, VPA; **seizure-free**	normal	none (slow acquisition of skills)	No	strabismus	abnormal with mainly left sided-spiking and slowing	MRI: normal	*UNC80* compound heterozygous: c.1694T>C, (p.Val565Ala) and c.8978G>A,(p.Arg2993Gln), rs371593882 (AR, LP/VUS); COBLL1: c.997-1G>A (AD, de novo, LP/VUS)	Mixed focal and generalised epilepsy	Significance uncertain
EE48/M	unknown	spasms, frequent myoclonic drop attacks and focal seizures 20-100/day	**VPA, CBZ, CLB, CBD oi**l, KD; **controlled** (clinical seizures only with intercurrent illness)	Severe ID, hypotor	frequent drop attacks, reduced function, less head control	No	very poor sleep pattern	slow background, generalised, disorganised multifocal spike and wave discharges, hypsarrythmia.	paucity of white matte	15q11.2-q13 deletion	Angelman Syndrome	Diagnosis and genetic counselling. MDT support for ongoing management.
EE57/F	unknown - recognised at 23 months	myoclonic jerks with arm raises 20-100/day	**VPA, KD** (partial improvement on KD but poorly tolerated otherwise); **refractory**	GDD from birth	severe GDD with microcephaly, dystonia, hyperreflexia, axial hypotonia, limb hypertonia	chorea, stereotypic hand movements	strabismus, cortical visual impairment, possible mild central disc coloboma, feeding difficulties, autistic features.	Burst suppression with frequent generalised and multifocal interictal epileptiform discharges, mainly as polyspikes.	Thinning corpus callosum; hypoplastic rostrum genu and body of corpus callosum, splenium absent; colpocephaly; giant cisterna magna; prominent sub-arachnoid spaces in frontal temporal parietal regions bilaterally.	*CDKL5* gene deletion on CMA: arr[GRCh37] Xp22.13 (17052902-19550265)x1, P	CDKL5 Deficiency Disorder	Diagnostic closure was critical for the family, who previously continued pursuing multiple specialist opinions. Also helped the local clinicians to maintain consistent, uninterrupted care.
EE61/M	6 months	febrile focal once/week; later focal, GTCS 10-19/day	**VPA**, **TPM, CBZ, CZP**, **PB**, **RIS,** KD; **refractory**	normal	neuroregression (speech and behaviour), hyperactivity, aggression, possible hypoxic insult associated with ICU admission for SE	No	1 x cafe-au-lait lesion	normal at presentation	CT, MRI: normal	*SCN1A:* c.2665delG(Ala878LeufsTer5), recurrent (rs1559200672), P, LoF	Dravet Syndrome (score: 9)	Earlier testing would have permitted earlier, targeted treatment. Genetic diagnosis became available after lost to follow-up, therefore unable to rationalise ASMs.
EE62/M	11 months	focal <5/day, triggered by tiredness	VPA, CLB; **seizure-free** (off ASMs)	possible language delay and hyperactive	mild to moderate DD (DQ 74): fine motor delay, mild speech delay, mild to moderate LD, hyperactive; normal gross motor skills	No	none	generalised polyspike and wave activity in sleep	MRI: delayed myelination	16p13.11 (gain), also present in mother; *KCNA2:* c.223G>A,(p.Glu75Lys), VUS but LP if de novo (paternal DNA unavailable for segregation).	DEE	Diagnostic closure, genetic counselling and cascade testing. The genetic results especially appreciated by the mother for future reproductive decisions.
EE63/M	9 months	initially febrile, GTS once a day; subsequently GTS at night, myoclonic, atonic, focal, atypical absence 5-9/day; triggered by fever and startle	**VPA, LTG, CZP**, CLB, KD as of 2018; **refractory**	normal	severe ID, autism, loss of milestones/arrest in development, focal neurological deficit: increased tone and brisk reflexes globally	No	premature; stormy neonatal course assumed due to hypoxic insult; excluded suspicion of storage disorder; visual impairment: poor fixing and following.	slow background, profoundly disrupted during sleep; frequent multifocal discharges of spike and polyspike semiology, mainly from biparietal regions.	MRI: HIE	*SCN8A:* c.1243G>A,(p.Glu415Lys), recurrent (PMID: 35230384), LP	LGS/*SCN8A* encephalopathy (DEE 13)	Cared for by grandparents who thought that issues related to the mother's very young age and poor health. Earlier testing would have permitted earlier, targeted treatment.

Suppl. Table 1 cont. **Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
EE64/M	3 weeks	focal, a few times/week (3 weeks). progressing to GTS <5/day; SE (PB loaded)	PB, **CBZ, VPA**; **controlled after adding CBZ**	normal	Global DD (DQ 16)	dystonia	daily "tik" exposure during pregnancy, cerebral palsy, severe GORD post PEG insertion	Initially normal EEG evolved ictal and interictal epileptiform activity, predominantly left temporal spike and waves	Ultrasound, CT, MRI: thinning corpus callosum, evidence of HIE.	*SCN2A:* c.656T>C,(p.Phe219Ser), LP; GoF (PMID: 35037686)	LGS spectrum / *SCN2A* encephalopathy	Excellent response and seizure control with CBZ. Earlier testing would have permitted earlier, targeted treatment. Diagnosis also important for the family, as redirected their assumption that the illness was related only to the mothers substance abuse in pregnancy.
EE65/M	6 months	spams <5 per day; periods of high spasm frequency	ACTH, VP; **seizure-free**	normal	regression with seizures but successfully normalised on ASMs	No	none	hypsarrhythmia	infancy but generalised white matter atrophy and thin corpus callosum at 10 years old.	16p13.11 (loss); present in the unaffected mother	16p13.11 microdeletion syndrome	Diagnostic closure, genetic counselling and cascade testing for the family.
EE66/F	5 months	focal (staring, nystagmoid eye movements, clenched jaw, hypotonic, apnoeic, cyanotic) 10-19/day; later focal refractory, mainly eye staring	**VPA, LEV, PB; seizure-free**	hopotonic, mild DD	profound GDD	No	transient tachypnoea of the newborn, feeding problems; dysmorphology: short palpebral fissures, 3rd fontanelle, frontal bossing; ocular disc pallor, strabismus, optic neuropathy.	excessive beta activity (which could be medication related) but also generalised background (2-3Hz delta)	MRI: White matter loss on post-natal MRI	1p36.33 (loss)	1p36 microdeletion syndrome	A complex early course and a considerable amount of time in hospital. Diagnostic closure and genetic counselling were very important to the anxious parents, who accessed multiple specialist opinions. MDT approach to targeted care.
EE79.1OYI/F	6 years	focal, GTS a few times/week, increasing to <5 per day	VPA, LTG, CZP, ketogenic diet - seizure-free (off ASMs)	regression noted at 21 months of age, rapidly progressing	rapid motor and cognitive regression; non-ambulant, non-verbal, sitting with support, reverted to nappies	hand wringing and washing movements	acquired microcephaly, mild thoracolumbar scoliosis	normal at presentation	CT: normal	*MECP2:* exonic deletion detected by clinical testing in another laboratory, no details available.	Rett Syndrome	Diagnostic closure and genetic counselling. MDT support for ongoing management.
EE87/F	5 months	febrile, focal, GTCS, hemiclonic 1 - 3 times/month, progressing to a few/week, SE (intubated and hospital-admitted)	**VPA**, CBZ, PB, LEV, CLB, **LTG, TPM**, PN, Biotin, Folinic Acid**; refractory**	normal	progressive regression (DQ 60), ADHD	No	none	generalised slowing	CT, MRI: normal	*SCN1A:* c.1178G>A, (p.Arg393His), recurrent (ClinVar ID: 68506; PMID: 12754708; 23195492, 22780858, 28544625; 35037686, rs121917927), LP, LoF	Dravet syndrome (score 12). SE and eventual demise.	Clinical reassessment and a revised diagnosis with implications for treatment. Earlier testing would have permitted earlier, targeted treatment and possibly, a better outcome. Died in ICU with systemic decompensation and liver dysfunction. Severe progressive course, unrelated to genetic variant.
EE89/F	2years 6months	not recorded	VPA, CLB, LTG; **lost to follow-up**	unknown	unknown	unknown	unknown	no record	no record	13q13.3del (loss o*f NBEA*)	DEE	Diagnostic closure and genetic counselling.
EE91/M	unknown - initialy seen at 1 yoa.	atonic head drops	KD; **good response** but presented after 1 yoa, so delayed opportunity for intervention	DD	DD/moderate ID	mixed movement disorder	CSF/serum glucose ratio 0.39, CSF lactate low 0.9.	normal	none	*SLC2A1:* c.49G>A,(p.Gly17Arg), recurrent (rs1345986424; ClinVar ID 597357), de novo, P	GLUT1 deficiency syndrome	Clinical suspicion of GLUT1 deficiency in this private healthcare patient. Improved on KD but developmentally delayed. Earlier genetic diagnosis would possibly have improved outcome.
EE99/F	16 months	GTCS 1-3/month, subsequently FMS <1/month; SE (hospital-admitted)	PB, **VPA** (failed weaning); **refractory**	mild delay: persistent head lag, delayed walking, language delay	DD (DQ 60): delayed walking, lost and regained ambulation, neuroregression with loss of skills.	waddling gait; hand movements	none	normal	CT, MRI: global atrophy, delayed myelination, thinning corpus callosum,	*SMC1A* :c.3285+1G>A, recurrent (PMID: 31334757), LP, LoF	DEE	Diagnostic closure and genetic counselling. Better clinical prognostic expectations related to gene encephalopathy
EIEE12/M	2 weeks	generalised myoclonic <5/day	ACTH, **VPA; seizure-free**	normal	none	No	none	independent left hemispheric discharges. Modified hypsarrhythmia.	CT: normal	16p13.11 (loss), unknown origin	16p13.11 microdeletion syndrome	Diagnosis and genetic counselling.

Suppl. Table 1 cont**. Clinical Information for patients with identified P/LP variants and VUS of interest.**

Pt no/sex	age at seizure onset	seizure description and frequency	ASMs and response (current ASM in bold)	development before seizure onset	subsequent development and neurological sequelae	gait/movement abnormality	other clinical features/conditions	EEG	imaging: findings	implicated variants[a]	Final diagnosis	Clinical utility of the genetic findings.
EIEE16/F	1st day of life	focal as a neonate, subsequently GTCS, myoclonic, multifocal/daily; SE	pyridoxine (stopped), biotin (stopped), **CBZ, VALP, CZP**, LEV; **refractory**	GDD	severe GDD and autism	wide based and slow gait, tremor, dystonia, choreoathetosis, repetitive movements.	non-specific dysmorphic features: flattened maxillae, protruding mandible, prominent eyes; inverted and flat feet.	generalized encephalopathy with temporal lobe epileptiform dysfunction; ictal activity with occipital onset spike and wave.	cerebellar atrophy	*CACNA1A:* c.2134G>A,(p.Ala712Thr), recurrent (PMID:27476654; 23934111; rs886037945), P, GoF	DEE / *CACNA1A* gene encephalopathy	Likely implications for care due to the delay in accessing a diagnosis. Lost to the system.
EIEE19/M	3 weeks	GTCS, myoclonic <5/day; later GTS <5 per day	VPA, **LTG; controlled**	normal	none	hand stereotypy	Dysmorphology: plagiocephaly (flattened right side), coarse facial features, cerebral palsy.	normal	none	*STXBP1:* c.1630G>C,(p.Gly544Arg), recurrent (ClinVar ID: 952587; PMID: 29314583, 28628100, 26514728, 23409955), de novo, P	DEE / *STXBP1* gene encephalopathy	Genetic diagnosis and extensive counselling very important for the parents, for closure and understanding the reason for their child's illness (previously told the epilepsy was due to HIE and CP).
EIEE2/F	1 month	GTCS 1-3/month, evolving to generalised spasms, myoclonic jerks once/day	PB, **VPA**, CZP, VGT, **LTG**, PN, improved on KD but unable to sustain financially; **refractory**	normal	profound DD, hypotonic, unable to roll or sit, fully dependent	myoclonic jerks	cortical visual impairment (not fixing and following) and hearing impairment.	generalised slowing; electrical SE	MRI: evidence of HIE	*CDKL5:* c.403+2T>A, LP	CDKL5 Deficiency Disorder	The previously assumed diagnosis of HIE did not align with the clinical course and setting. The genetic diagnosis facilitated understanding and improved prognostic expectations.
EIEE3/M	2 months	focal (multifocal jerking) <5/day; progressing to generalised myoclonic and epileptic spasms a few/week	**VPA, PB,** PN**,** VGT**; refractory**	normal	severe GDD and neuroregression at 6 months	spontaneous limb movements against gravity	visual impairment: does not fix and follow, normal fundoscopy but abnormal VEP at 2months,	generalised slowing with burst-suppression	MRI: Global atrophy, evidence of HIE	*KCNT1:* c.862G>A,(p.Gly288Ser); recurrent (ClinVar ID: 126421; PMID:24029078; 32167590, 29196579; rs587777264), P, GoF	DEE - probable Early Infantile Migrating Focal Seizures/*KCNT1* encephalopathy	Earlier genetic diagnosis would have supported targeted therapy e.g., quinidine

[a] de novo occurrence stated if established. In many cases, paternal DNA was not available for segregation analysis. More variant details in Table S2.

[b] PMID: 30321769 - detected during our published pilot study

ACTH: adrenocorticotropic hormone; AR: autosomal recessive; ASD: atrial septal defect; ASM: anti-seizure medication; CBD: Cannabidiol CBZ: Carbamazepine; CLB: Clobazam; CMA: chromosomal microarray; CP: cerebral palsy; CSF: cerebro-spinal fluid; CZP: Clonazepam; DD: developmental delay; EIEE: early-infantile epileptic encephalopathy; EOEE: early-onset epileptic encephalopathy; ES: epileptic spasms; ESM: Ethosuximide; FMS: focal motor seizures; GDD: global developmental delay; GoF: gain of function; GORD: gastro-oesophageal reflux disorder; GTS: generalised tonic seizures; GTCS: generalised tonic-clinic seizures; HIE: hypoxic-ischemic encephalopathy; ICU: intensive care unit; ID: intellectual disability; KD: ketogenic diet; LEV: Levetiracetam; LGS: Lennox-Gestaut Syndrome; LoF: loss of function; LP: likely pathogenic; LSEN: Learners with Special Education Needs; LTG: Lamotrigine; MDT: multidisciplinary team; P: pathogenic; PB: Phenobarbital; PHT: Phenytoin; PN: Pyridoxine; RFM: Rufinamide; RIS: Risperidone; STP: Stiripentol; tik: South African street name for crystal methamphetamine; TPM: Topiramate; VGT: Vigabatrin; VNS: vagus nerve stimulation; VPA: Sodium Valproate; VUS: variant of uncertain significance; yoa: year/s of age.

Suppl. Table 2**. Summary Statistics Comparing the Clinical Characteristics of Patients with Candidate SNVs/indels and patients with No Detected Candidate SNVs/indels.**

SNV	**All patients** = 234[1]	**Patients with no candidate SNVs/indels** N = 196[1]	**Patients with candidate SNV/indels**[a] N = 38[1]	**p-value**[2]
Sex				0.5
Male	122 (52%)	104 (53%)	18 (47%)	
Female	112 (48%)	92 (47%)	20 (53%)	
ethnicity				0.002
European	17 (8.1%)	8 (4.6%)	9 (25%)	
Indigenous Black African	102 (48%)	90 (51%)	12 (33%)	
Mixed Ancestry	90 (43%)	75 (43%)	15 (42%)	
Asian	1 (0.5%)	1 (0.6%)	0 (0%)	
other	1 (0.5%)	1 (0.6%)	0 (0%)	
Unknown	23	21	2	
seizure type at onset: febrile	39 (17%)	30 (15%)	9 (24%)	0.2
seizure type at onset: focal	57 (24%)	40 (20%)	17 (45%)	0.001
seizure type at onset: hemiclonic	3 (1.3%)	1 (0.5%)	2 (5.3%)	0.069
seizure type at onset: spasms	33 (14%)	30 (15%)	3 (7.9%)	0.2
seizure type at onset: other	21 (9.0%)	18 (9.2%)	3 (7.9%)	>0.9
seizure type at onset: generalised	98 (42%)	77 (39%)	21 (55%)	0.068
seizure type at onset: generalised tonic	20 (8.5%)	17 (8.7%)	3 (7.9%)	>0.9
seizure type at onset: generalised clonic	4 (1.7%)	2 (1.0%)	2 (5.3%)	0.12
seizure type at onset: generalised tonic-clonic	70 (30%)	54 (28%)	16 (42%)	0.073
seizure type at onset: generalised myoclonic	19 (8.1%)	15 (7.7%)	4 (11%)	0.5
seizure type at onset: generalised absence	2 (0.9%)	1 (0.5%)	1 (2.6%)	0.3
seizure type at onset: generalised atonic	7 (3.0%)	7 (3.6%)	0 (0%)	0.6
seizure type at onset: generalised other	4 (1.7%)	3 (1.5%)	1 (2.6%)	0.5
current seizure type: febrile	17 (7.3%)	13 (6.6%)	4 (11%)	0.5
current seizure type: focal	64 (27%)	46 (23%)	18 (47%)	0.002
current seizure type: hemiclonic	3 (1.3%)	0 (0%)	3 (7.9%)	0.004
current seizure type: spasms	7 (3.0%)	6 (3.1%)	1 (2.6%)	>0.9
current seizure type: other	11 (4.7%)	7 (3.6%)	4 (11%)	0.083
current seizure type: generalised	116 (50%)	92 (47%)	24 (63%)	0.067
current seizure type: generalised tonic	42 (18%)	34 (17%)	8 (21%)	0.6
current seizure type: generalised clonic	6 (2.6%)	4 (2.0%)	2 (5.3%)	0.3
current seizure type: generalised tonic-	83 (35%)	69 (35%)	14 (37%)	0.8

SNV	All patients = 234[1]	Patients with no candidate SNVs/indels N = 196[1]	Patients with candidate SNV/indels[a] N = 38[1]	p-value[2]
clonic				
current seizure type: generalised myoclonic	53 (23%)	39 (20%)	14 (37%)	0.022
current seizure type: generalised absence	18 (7.7%)	14 (7.1%)	4 (11%)	0.5
current seizure type: generalised atonic	22 (9.4%)	18 (9.2%)	4 (11%)	0.8
current seizure type: other	10 (4.3%)	9 (4.6%)	1 (2.6%)	>0.9
seizure trigger	81 (40%)	62 (38%)	19 (53%)	0.092
status epilepticus (SE)	84 (41%)	62 (37%)	22 (59%)	0.012
developmental delay before seizure onset	**58 (29%)**	**50 (30%)**	**8 (21%)**	**0.3**
gait disorder before seizure onset	9 (4.3%)	7 (4.0%)	2 (5.6%)	0.7
impaired motor dev. before seizure onset	35 (17%)	32 (19%)	3 (8.3%)	0.12
cognitive delay prior to seizure onset	33 (17%)	28 (18%)	5 (14%)	0.6
neuro-regression	83 (39%)	65 (37%)	18 (47%)	0.2
neuro-regression associated with poor seizure control/clustering	60 (77%)	47 (76%)	13 (81%)	0.8
family history of seizures	54 (25%)	42 (24%)	12 (32%)	0.3
family history of febrile seizures	10 (4.8%)	7 (4.0%)	3 (8.6%)	0.4
family history of developmental problems	7 (3.4%)	6 (3.6%)	1 (2.8%)	>0.9
dysmorphic features	**26 (11%)**	**19 (9.7%)**	**7 (18%)**	**0.2**
visual impairment	29 (13%)	23 (13%)	6 (16%)	0.6
focal neurological deficit	26 (12%)	21 (12%)	5 (14%)	0.8
ophthalmological abnormalities	16 (7.5%)	13 (7.3%)	3 (8.1%)	0.7
normal EEG background	104 (49%)	81 (47%)	23 (62%)	0.085
evolving EEG background	49 (46%)	34 (43%)	15 (54%)	0.3
interictal epileptiform activity	96 (45%)	82 (47%)	14 (38%)	0.3
recorded ictal activity	62 (30%)	48 (28%)	14 (39%)	0.2
None of the following	157 (67%)	127 (65%)	30 (79%)	0.089
Hypsarrhythmia	18 (7.7%)	18 (9.2%)	0 (0%)	0.050
Burst-suppression	22 (9.4%)	20 (10%)	2 (5.3%)	0.5
Electro-decrements	5 (2.1%)	4 (2.0%)	1 (2.6%)	0.6
modified hypsarrhythmia	9 (3.8%)	8 (4.1%)	1 (2.6%)	>0.9
other EEG patterns	13 (5.6%)	12 (6.1%)	1 (2.6%)	0.7
Abnormality on brain imaging	89 (44%)	74 (45%)	15 (42%)	0.7
Structural brain anomalies	**12 (5.1%)**	**8 (4.1%)**	**4 (11%)**	**0.11**
Global atrophy	18 (7.7%)	13 (6.6%)	5 (13%)	0.2
Delayed myelination	3 (1.3%)	1 (0.5%)	2 (5.3%)	0.069
Corpus callosum thinning	11 (4.7%)	7 (3.6%)	4 (11%)	0.083
Focal cortical dysplasia	5 (2.1%)	5 (2.6%)	0 (0%)	>0.9

SNV	All patients = 234[1]	Patients with no candidate SNVs/indels N = 196[1]	Patients with candidate SNV/indels[a] N = 38[1]	p-value[2]
evidence of hypoxic-ischemic encephalopathy (HIE)	17 (7.3%)	11 (5.6%)	6 (16%)	0.039
Calcification	3 (1.3%)	3 (1.5%)	0 (0%)	>0.9
other findings on imaging	58 (25%)	51 (26%)	7 (18%)	0.3
movement abnormality	**39 (17%)**	**21 (11%)**	**18 (47%)**	**<0.001**
movement disorder: chorea	2 (0.9%)	1 (0.5%)	1 (2.6%)	0.3
movement disorder: dystonia	6 (2.6%)	5 (2.6%)	1 (2.6%)	>0.9
movement disorder: stereotypy	7 (3.0%)	5 (2.6%)	2 (5.3%)	0.3
movement disorder: tremor	1 (0.4%)	0 (0%)	1 (2.6%)	0.2
movement disorder: other	4 (1.7%)	1 (0.5%)	3 (7.9%)	0.014
crouched gait	7 (3.3%)	4 (2.3%)	3 (8.1%)	0.10
sleep cycle disturbance	14 (6.6%)	10 (5.7%)	4 (11%)	0.3
None of the following psych disorders	129 (55%)	111 (57%)	18 (47%)	0.3
Hyperactivity	44 (19%)	34 (17%)	10 (26%)	0.2
Depression	1 (0.4%)	0 (0%)	1 (2.6%)	0.2
Attention difficulties	**21 (9.0%)**	**13 (6.6%)**	**8 (21%)**	**0.010**
Autism spectrum	**30 (13%)**	**21 (11%)**	**9 (24%)**	**0.036**
other psychiatric disorders	8 (3.4%)	8 (4.1%)	0 (0%)	0.4
behavioural problems	61 (31%)	46 (28%)	15 (43%)	0.093
tb	5 (2.2%)	4 (2.1%)	1 (2.7%)	>0.9
tbi	4 (1.8%)	3 (1.6%)	1 (2.7%)	0.5
age_1st_seizure	8 (3, 18)	9 (4, 18)	5 (2, 9)	0.015

[1]n (%); Median (IQR) [2]Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test
[a]This total does not include two individuals with SNVs of uncertain significance and pathogenic CNVs.

Suppl. Table 3. **Summary Statistics Comparing the Clinical Characteristics of Patients with Candidate CNVs and Patients with no Detected Candidate CNVs.**

CNV	**All patients** N = 234[1]	**Patients with no candidate CNVs** N = 221[1]	**Patients with candidate CNVs** N = 13[1]	**p-value**[2]
Sex				0.9
Male	122 (52%)	115 (52%)	7 (54%)	
Female	112 (48%)	106 (48%)	6 (46%)	
ethnicity				0.2
European	17 (8.1%)	15 (7.5%)	2 (17%)	
Indigenous Black African	102 (48%)	99 (50%)	3 (25%)	
Mixed Ancestry	90 (43%)	83 (42%)	7 (58%)	
Asian	1 (0.5%)	1 (0.5%)	0 (0%)	
other	1 (0.5%)	1 (0.5%)	0 (0%)	
Unknown	23	22	1	
seizure type at onset: febrile	39 (17%)	38 (17%)	1 (7.7%)	0.7
seizure type at onset: focal	57 (24%)	55 (25%)	2 (15%)	0.7
seizure type at onset: hemiclonic	3 (1.3%)	3 (1.4%)	0 (0%)	>0.9
seizure type at onset: spasms	33 (14%)	31 (14%)	2 (15%)	>0.9
seizure type at onset: other	21 (9.0%)	19 (8.6%)	2 (15%)	0.3
seizure type at onset: generalised	98 (42%)	94 (43%)	4 (31%)	0.4
seizure type at onset: generalised tonic	20 (8.5%)	19 (8.6%)	1 (7.7%)	>0.9
seizure type at onset: generalised clonic	4 (1.7%)	4 (1.8%)	0 (0%)	>0.9
seizure type at onset: generalised tonic-clonic	70 (30%)	67 (30%)	3 (23%)	0.8
seizure type at onset: generalised myoclonic	19 (8.1%)	17 (7.7%)	2 (15%)	0.3
seizure type at onset: generalised absence	2 (0.9%)	2 (0.9%)	0 (0%)	>0.9
seizure type at onset: generalised atonic	7 (3.0%)	7 (3.2%)	0 (0%)	>0.9
seizure type at onset: generalised other	4 (1.7%)	4 (1.8%)	0 (0%)	>0.9
current seizure type: febrile	17 (7.3%)	16 (7.2%)	1 (7.7%)	>0.9
current seizure type: focal	64 (27%)	62 (28%)	2 (15%)	0.5
current seizure type: hemiclonic	3 (1.3%)	3 (1.4%)	0 (0%)	>0.9
current seizure type: spasms	7 (3.0%)	7 (3.2%)	0 (0%)	>0.9
current seizure type: other	11 (4.7%)	10 (4.5%)	1 (7.7%)	0.5
current seizure type: generalised	116 (50%)	108 (49%)	8 (62%)	0.4
current seizure type: generalised tonic	42 (18%)	41 (19%)	1 (7.7%)	0.5
current seizure type: generalised clonic	6 (2.6%)	6 (2.7%)	0 (0%)	>0.9
current seizure type: generalised tonic-clonic	83 (35%)	79 (36%)	4 (31%)	>0.9

CNV	All patients N = 234[1]	Patients with no candidate CNVs N = 221[1]	Patients with candidate CNVs N = 13[1]	p-value[2]
current seizure type: generalised myoclonic	53 (23%)	48 (22%)	5 (38%)	0.2
current seizure type: generalised absence	18 (7.7%)	18 (8.1%)	0 (0%)	0.6
current seizure type: generalised atonic	22 (9.4%)	19 (8.6%)	3 (23%)	0.11
current seizure type: other	10 (4.3%)	10 (4.5%)	0 (0%)	>0.9
seizure trigger	81 (40%)	75 (39%)	6 (55%)	0.4
status epilepticus (SE)	84 (41%)	82 (42%)	2 (20%)	0.2
developmental delay before seizure onset	**58 (29%)**	**51 (27%)**	**7 (64%)**	**0.014**
gait disorder before seizure onset	9 (4.3%)	9 (4.5%)	0 (0%)	>0.9
impaired motor dev. before seizure onset	35 (17%)	29 (15%)	6 (60%)	0.002
cognitive delay prior to seizure onset	33 (17%)	27 (15%)	6 (60%)	0.002
neuro-regression	83 (39%)	79 (39%)	4 (33%)	0.8
neuro-regression associated with poor seizure control/clustering	60 (77%)	57 (77%)	3 (75%)	>0.9
family history of seizures	54 (25%)	52 (26%)	2 (18%)	0.7
family history of febrile seizures	10 (4.8%)	9 (4.6%)	1 (9.1%)	0.4
family history of developmental problems	7 (3.4%)	7 (3.6%)	0 (0%)	>0.9
dysmorphic features	**26 (11%)**	**20 (9.0%)**	**6 (46%)**	**0.001**
visual impairment	29 (13%)	26 (13%)	3 (25%)	0.2
focal neurological deficit	26 (12%)	24 (12%)	2 (17%)	0.6
ophthalmological abnormalities	16 (7.5%)	13 (6.4%)	3 (27%)	0.039
normal EEG background	104 (49%)	98 (49%)	6 (55%)	0.7
evolving EEG background	49 (46%)	47 (46%)	2 (50%)	>0.9
interictal epileptiform activity	96 (45%)	92 (46%)	4 (33%)	0.4
recorded ictal activity	62 (30%)	59 (30%)	3 (27%)	>0.9
None of the following	157 (67%)	149 (67%)	8 (62%)	0.8
Hypsarrhythmia	18 (7.7%)	15 (6.8%)	3 (23%)	0.067
Burst-suppression	22 (9.4%)	21 (9.5%)	1 (7.7%)	>0.9
Electro-decrements	5 (2.1%)	5 (2.3%)	0 (0%)	>0.9
modified hypsarrhythmia	9 (3.8%)	9 (4.1%)	0 (0%)	>0.9
other EEG patterns	13 (5.6%)	13 (5.9%)	0 (0%)	>0.9
Abnormality on brain imaging	89 (44%)	82 (44%)	7 (54%)	0.6
Structural brain anomalies	**12 (5.1%)**	**7 (3.2%)**	**5 (38%)**	**<0.001**
Global atrophy	18 (7.7%)	16 (7.2%)	2 (15%)	0.3
Delayed myelination	3 (1.3%)	2 (0.9%)	1 (7.7%)	0.2
Corpus callosum thinning	11 (4.7%)	7 (3.2%)	4 (31%)	0.002
Focal cortical dysplasia	5 (2.1%)	5 (2.3%)	0 (0%)	>0.9
evidence of hypoxic-ischemic encephalopathy (HIE)	17 (7.3%)	16 (7.2%)	1 (7.7%)	>0.9

CNV	All patients N = 234[1]	Patients with no candidate CNVs N = 221[1]	Patients with candidate CNVs N = 13[1]	p-value[2]
Calcification	3 (1.3%)	3 (1.4%)	0 (0%)	>0.9
other findings on imaging	58 (25%)	52 (24%)	6 (46%)	0.094
movement abnormality	**39 (17%)**	**35 (16%)**	**4 (31%)**	**0.2**
movement disorder: chorea	2 (0.9%)	1 (0.5%)	1 (7.7%)	0.11
movement disorder: dystonia	6 (2.6%)	4 (1.8%)	2 (15%)	0.038
movement disorder: stereotypy	7 (3.0%)	6 (2.7%)	1 (7.7%)	0.3
movement disorder: tremor	1 (0.4%)	1 (0.5%)	0 (0%)	>0.9
movement disorder: other	4 (1.7%)	3 (1.4%)	1 (7.7%)	0.2
crouched gait	7 (3.3%)	7 (3.4%)	0 (0%)	>0.9
sleep cycle disturbance	14 (6.6%)	12 (6.0%)	2 (18%)	0.2
None of the following psych disorders	129 (55%)	121 (55%)	8 (62%)	0.6
Hyperactivity	44 (19%)	42 (19%)	2 (15%)	>0.9
Depression	1 (0.4%)	1 (0.5%)	0 (0%)	>0.9
Attention difficulties	**21 (9.0%)**	**21 (9.5%)**	**0 (0%)**	**0.6**
Autism spectrum	**30 (13%)**	**28 (13%)**	**2 (15%)**	**0.7**
other psychiatric disorders	8 (3.4%)	7 (3.2%)	1 (7.7%)	0.4
behavioural problems	61 (31%)	58 (31%)	3 (27%)	>0.9
tb	5 (2.2%)	5 (2.4%)	0 (0%)	>0.9
tbi	4 (1.8%)	3 (1.4%)	1 (7.7%)	0.2
age_1st_seizure	8 (3, 18)	8 (3, 18)	7 (6, 18)	0.7

[1]n (%); Median (IQR) [2]Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

Suppl. Table 4. **Summary Statistics Comparing the Clinical Characteristics of Patients with Candidate Variants (SNVs/indels and CNVs) and Patients with No Detected Candidate Variants.**

All Variants	**All patients** N = 234[1]	**Patients with no candidate SNVs/indels or CNVs** N = 183[1]	**Patients with candidate SNVs/indels and CNVs** N = 51[1]	**p-value**[2]
Sex				0.6
Male	122 (52%)	97 (53%)	25 (49%)	
Female	112 (48%)	86 (47%)	26 (51%)	
ethnicity				<0.001
European	17 (8.1%)	6 (3.7%)	11 (23%)	
Indigenous Black African	102 (48%)	87 (53%)	15 (31%)	
Mixed Ancestry	90 (43%)	68 (42%)	22 (46%)	
Asian	1 (0.5%)	1 (0.6%)	0 (0%)	
other	1 (0.5%)	1 (0.6%)	0 (0%)	
Unknown	23	20	3	
seizure type at onset: febrile	39 (17%)	29 (16%)	10 (20%)	0.5
seizure type at onset: focal	57 (24%)	38 (21%)	19 (37%)	0.015
seizure type at onset: hemiclonic	3 (1.3%)	1 (0.5%)	2 (3.9%)	0.12
seizure type at onset: spasms	33 (14%)	28 (15%)	5 (9.8%)	0.3
seizure type at onset: other	21 (9.0%)	16 (8.7%)	5 (9.8%)	0.8
seizure type at onset: generalised	98 (42%)	73 (40%)	25 (49%)	0.2
seizure type at onset: generalised tonic	20 (8.5%)	16 (8.7%)	4 (7.8%)	>0.9
seizure type at onset: generalised clonic	4 (1.7%)	2 (1.1%)	2 (3.9%)	0.2
seizure type at onset: generalised tonic-clonic	70 (30%)	51 (28%)	19 (37%)	0.2
seizure type at onset: generalised myoclonic	19 (8.1%)	13 (7.1%)	6 (12%)	0.4
seizure type at onset: generalised absence	2 (0.9%)	1 (0.5%)	1 (2.0%)	0.4
seizure type at onset: generalised atonic	7 (3.0%)	7 (3.8%)	0 (0%)	0.4
seizure type at onset: generalised other	4 (1.7%)	3 (1.6%)	1 (2.0%)	>0.9
current seizure type: febrile	17 (7.3%)	12 (6.6%)	5 (9.8%)	0.5
current seizure type: focal	64 (27%)	44 (24%)	20 (39%)	0.032
current seizure type: hemiclonic	3 (1.3%)	0 (0%)	3 (5.9%)	0.010
current seizure type: spasms	7 (3.0%)	6 (3.3%)	1 (2.0%)	>0.9
current seizure type: other	11 (4.7%)	6 (3.3%)	5 (9.8%)	0.065

All Variants	**All patients** N = 234[1]	**Patients with no candidate SNVs/indels or CNVs** N = 183[1]	**Patients with candidate SNVs/indels and CNVs** N = 51[1]	**p-value**[2]
current seizure type: generalised	116 (50%)	84 (46%)	32 (63%)	0.033
current seizure type: generalised tonic	42 (18%)	33 (18%)	9 (18%)	>0.9
current seizure type: generalised clonic	6 (2.6%)	4 (2.2%)	2 (3.9%)	0.6
current seizure type: generalised tonic-clonic	83 (35%)	65 (36%)	18 (35%)	>0.9
current seizure type: generalised myoclonic	53 (23%)	34 (19%)	19 (37%)	0.005
current seizure type: generalised absence	18 (7.7%)	14 (7.7%)	4 (7.8%)	>0.9
current seizure type: generalised atonic	22 (9.4%)	15 (8.2%)	7 (14%)	0.3
current seizure type: other	10 (4.3%)	9 (4.9%)	1 (2.0%)	0.7
seizure trigger	81 (40%)	56 (36%)	25 (53%)	0.040
status epilepticus (SE)	84 (41%)	60 (38%)	24 (51%)	0.11
developmental delay before seizure onset	**58 (29%)**	**43 (28%)**	**15 (31%)**	**0.7**
gait disorder before seizure onset	9 (4.3%)	7 (4.3%)	2 (4.3%)	
impaired motor dev. before seizure onset	35 (17%)	26 (16%)	9 (20%)	0.6
cognitive delay prior to seizure onset	33 (17%)	22 (15%)	11 (24%)	0.14
neuro-regression	83 (39%)	61 (37%)	22 (44%)	0.4
neuro-regression associated with poor seizure control/clustering	60 (77%)	44 (76%)	16 (80%)	>0.9
family history of seizures	54 (25%)	40 (24%)	14 (29%)	0.5
family history of febrile seizures	10 (4.8%)	6 (3.7%)	4 (8.7%)	0.2
family history of developmental problems	7 (3.4%)	6 (3.8%)	1 (2.1%)	>0.9
dysmorphic features	**26 (11%)**	**13 (7.1%)**	**13 (25%)**	**<0.001**
visual impairment	29 (13%)	20 (12%)	9 (18%)	0.3
focal neurological deficit	26 (12%)	19 (11%)	7 (14%)	0.6
ophthalmological abnormalities	16 (7.5%)	10 (6.0%)	6 (12%)	0.2
normal EEG background	104 (49%)	75 (46%)	29 (60%)	0.079
evolving EEG background	49 (46%)	32 (43%)	17 (53%)	0.3
interictal epileptiform activity	96 (45%)	78 (48%)	18 (37%)	0.2
recorded ictal activity	62 (30%)	45 (28%)	17 (36%)	0.3
None of the following	157 (67%)	119 (65%)	38 (75%)	0.2
Hypsarrhythmia	18 (7.7%)	15 (8.2%)	3 (5.9%)	0.8
Burst-suppression	22 (9.4%)	19 (10%)	3 (5.9%)	0.4

All Variants	**All patients** N = 234[1]	**Patients with no candidate SNVs/indels or CNVs** N = 183[1]	**Patients with candidate SNVs/indels and CNVs** N = 51[1]	**p-value**[2]
Electro-decrements	5 (2.1%)	4 (2.2%)	1 (2.0%)	>0.9
modified hypsarrhythmia	9 (3.8%)	8 (4.4%)	1 (2.0%)	0.7
other EEG patterns	13 (5.6%)	12 (6.6%)	1 (2.0%)	0.3
Abnormality on brain imaging	89 (44%)	67 (44%)	22 (45%)	>0.9
Structural brain anomalies	**12 (5.1%)**	**3 (1.6%)**	**9 (18%)**	**<0.001**
Global atrophy	18 (7.7%)	11 (6.0%)	7 (14%)	0.078
Delayed myelination	3 (1.3%)	0 (0%)	3 (5.9%)	0.010
Corpus callosum thinning	11 (4.7%)	3 (1.6%)	8 (16%)	<0.001
Focal cortical dysplasia	5 (2.1%)	5 (2.7%)	0 (0%)	0.6
evidence of hypoxic-ischemic encephalopathy (HIE)	17 (7.3%)	10 (5.5%)	7 (14%)	0.063
Calcification	3 (1.3%)	3 (1.6%)	0 (0%)	>0.9
other findings on imaging	58 (25%)	45 (25%)	13 (25%)	0.9
movement abnormality	**39 (17%)**	**17 (9.3%)**	**22 (43%)**	**<0.001**
movement disorder: chorea	2 (0.9%)	0 (0%)	2 (3.9%)	0.047
movement disorder: dystonia	6 (2.6%)	3 (1.6%)	3 (5.9%)	0.12
movement disorder: stereotypy	7 (3.0%)	4 (2.2%)	3 (5.9%)	0.2
movement disorder: tremor	1 (0.4%)	0 (0%)	1 (2.0%)	0.2
movement disorder: other	4 (1.7%)	0 (0%)	4 (7.8%)	0.002
crouched gait	7 (3.3%)	4 (2.4%)	3 (6.2%)	0.2
sleep cycle disturbance	14 (6.6%)	8 (4.9%)	6 (13%)	0.089
None of the following psych disorders	129 (55%)	103 (56%)	26 (51%)	0.5
Hyperactivity	44 (19%)	32 (17%)	12 (24%)	0.3
Depression	1 (0.4%)	0 (0%)	1 (2.0%)	0.2
Attention difficulties	**21 (9.0%)**	**13 (7.1%)**	**8 (16%)**	**0.091**
Autism spectrum	**30 (13%)**	**19 (10%)**	**11 (22%)**	**0.035**
other psychiatric disorders	8 (3.4%)	7 (3.8%)	1 (2.0%)	>0.9
behavioural problems	61 (31%)	43 (28%)	18 (39%)	0.2
tb	5 (2.2%)	4 (2.3%)	1 (2.0%)	>0.9
tbi	4 (1.8%)	2 (1.2%)	2 (4.0%)	0.2
age_1st_seizure	8 (3, 18)	9 (4, 18)	6 (3, 10)	0.042

[1]n (%); Median (IQR) [2]Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

Suppl. Table 5. **Genetic Variants detected with the gene panel, ES and CMA (P/LP and VUS).**

pt.	genomic location (Hg19)	gene/s	variant type	coding DNA change (HGVS)	AA change	LoF/GoF*	zygosity	inheritance	ACMG classification	recurrent/novel	Detection method
EE54	chr8:g.108348477A>C	*ANGPT1*	missense	c.476T>G	Leu159Arg	-	het	de novo	VUS	novel	ES
EE34	chr19:g.1469194C>A	*APC2*	missense	c.5894C>A	Ala1965Glu	-	comp het	AR	VUS	rs1038929926	ES
	chr19:g.1468284G>A	*APC2*	missense	c.4984G>A	Ala1662Thr	-	comp het	AR	VUS	rs373455264	ES
EE27	chr1:g.160100390 A>G	*ATP1A2*	splice region	c.1827+3A>G	-	LoF	het	maternal	VUS	rs377238291	DEE panel
EIEE16	chr19:g.13414398C>T	*CACNA1A*	missense	c.2134G>A	Ala712Thr	GoF (PMID: 31468518)	het	de novo	P	recurrent (rs886037945)	DEE panel
EE57	arr[hg19] Xp22.13 (17052902-19550265)x1	*CDKL5*	CNV: loss	-	-	-	het	not established **	LP	non-recurrent	CMA and MLPA
EIEE2	chrX:g.18598090T>A	*CDKL5*	splice donor	c.403+2T>A	-	LoF	het (female)	not established **	LP	novel	DEE panel
EE24	chr1:g.109811342G>A	*CELSR2*	missense	c.6458G>A	Arg2153Gln	-	comp het	AR	VUS	rs771675760	DEE panel
	chr1:g.109272890A>C	*CELSR2*	missense	c.8201A>C	Tyr2734Ser	-	comp het	AR	VUS	novel	ES
EE20	chr15:g.93510649C>T	*CHD2*	missense	c.2095C>T	Arg699Trp		het	not established **	LP	recurrent (ClinVar 429658; rs1131691515)	DEE panel
EE47	chr2:g.165561616C>T	*COBLL1*	splice acceptor	c.997-1G>A	-	LoF	het	de novo	VUS	novel (PMID: PMC6698801)	ES
	chr2:g.210846975G>A	*UNC80*	missense	c.8993G>A	Arg2998Gln	-	comp het	AR	VUS	rs371593882	ES
	chr2:g.210683717T>C	*UNC80*	missense/splice region	c.1694T>C	Val565Ala	-	comp het	AR	VUS	novel	ES
EE134	chr1:g.182357872T>C	*GLUL*	start lost	c.1A>G	Met1Val	-	het	de novo	VUS	novel (A>T change rs1131691970)	ES

Suppl. Table 5. cont. **Genetic Variants detected with the gene panel, ES and CMA (P/LP and VUS).**

pt.	genomic location (Hg19)	gene/s	variant type	coding DNA change (HGVS)	AA change	LoF/GoF*	zygosity	inheritance	ACMG classification	recurrent/novel	Detection method
EE62	chr1:g.111147182C>T	*KCNA2*	missense	c.223G>A	Glu75Lys	-	het	not established **	VUS (LP if de novo)	novel	DEE panel
	arr[hg19]16p13.11(15123951-16305677)x3	*NA*	CNV: gain	-	-	-	het	maternal	LP	recurrent	CMA and MLPA
EE127	chr9:g.138660769A>G	*KCNT1*	missense	c.1496A>G	His499Arg	-	het	de novo	P	recurrent (ClinVar ID: 449802; rs1554774362)	DEE panel
EIEE3	chr9:g.138651532G>A	*KCNT1*	missense	c.862G>A	Gly288Ser	GoF (PMID: 29196579)	het	not established **	P	recurrent (ClinVar ID: 126421; rs587777264)	DEE panel
EIEE12	16p13.11 deletion	*NA*	CNV: loss	-	-	-	het	not established **	LP	recurrent	CMA (clinical lab) and MLPA
EE79	exonic deletion	*MECP2*	likely frameshift	exonic deletion	-	LoF	het	not established	P	recurrent	MLPA (clinical lab)
EE113	chr18:g.55268931G>A	*NARS*	stop gained	c.1600C>T	Arg534Ter	LoF	het	de novo	P	recurrent (ClinVar ID: 982711; rs2051507892)	ES
EE11	chrX:g.99657626G>A	*PCDH19*	stop gained	c.2512C>T	Gln838Ter	LoF	het (female)	de novo	P	novel (PMID: 30321769)***	DEE panel
EE110	chr1:g.99771894delT	*PLPPR4*	frameshift	c.1620delT	-	LoF	het	de novo	VUS	novel	ES
DS1	chr2:g.166848438C>T	*SCN1A*	missense	c.5314G>A	Ala1772Thr	-	het	not established **	P	recurrent (ClinVar ID: 68570; rs121917980)	DEE panel
DS10	deletion of exons 5 - 8	*SCN1A*	frameshift	deletion exons 5-8	Tyr202Hisfs*10	LoF	het	not established **	P	PMID: 30321769***	DEE panel and MLPA

Suppl. Table 5. cont. **Genetic Variants detected with the gene panel, ES and CMA (P/LP and VUS).**

pt.	genomic location (Hg19)	gene/s	variant type	coding DNA change (HGVS)	AA change	LoF/GoF*	zygosity	inheritance	ACMG classification	recurrent/novel	Detection method
DS11	chr2:g.166895937C>G	*SCN1A*	missense	c.2552G>C	Arg851Pro	-	het	de novo	P	recurrent (ClinVar ID: 98593; rs121918785)	DEE panel
DS12	chr2:g.166859217A>C	*SCN1A*	missense	c.4016T>G	Val1339Gly	-	het	de novo	P	novel (PMID: 30321769)***	DEE panel
DS14	chr2:g.166848516C>A	*SCN1A*	missense	c.5236G>T	Gly1746Trp	LoF (PMID: 35037686)	het	not established **	LP	novel (PMID: 30321769)***	DEE panel
DS19	chr2:g.166854634delAAAGT	*SCN1A*	frameshift	c.4352_4356del	Tyr1451CysfsTer22	LoF	het	not established **	P	novel (PMID: 30321769)***	DEE panel
DS2	chr2:g.166892946delT	*SCN1A*	frameshift	c.3007delA	Ile1003LeufsTer10	LoF	het	not established **	P	novel (PMID: 30321769)***	DEE panel
DS22	chr2:g.166904178G>A	*SCN1A*	stop gained	c.1129C>T	Arg377Ter	LoF	het	not established **	P	recurrent (ClinVar ID: 189963; rs794726799)	DEE panel
DS4	chr2:g.166909392G>A	*SCN1A*	stop gained	c.664C>T	Arg222Ter	LoF	het	de novo	P	recurrent (ClinVar ID: 12889; rs121918624)	DEE panel
EE10	chr2:g.166852628G>A	*SCN1A*	splice acceptor	c.4444-1C>T	-	LoF	het	not established **	P	recurrent (ClinVar ID: 530456; rs1553521567)	DEE panel
EE12	chr2:g.166894396G>A	*SCN1A*	missense	c.2803C>T	Arg935Cys	LoF (PMID: 35037686)	het	not established **	P	recurrent (ClinVar ID: 68604; rs121918775)	DEE panel
EE21	chr2:g.166901695T>A	*SCN1A*	missense	c.1520A>T	Lys507Ile	-	het	not established **	VUS (LP if de novo)	novel	DEE panel

Suppl. Table 5. cont. **Genetic Variants detected with the gene panel, ES and CMA (P/LP and VUS).**

pt.	genomic location (Hg19)	gene/s	variant type	coding DNA change (HGVS)	AA change	LoF/GoF*	zygosity	inheritance	ACMG classification	recurrent/novel	Detection method
EE61	chr2:g.166894566delC	*SCN1A*	frameshift	c.2665delG	Ala878LeufsTer5	LoF	het	not established**	P	recurrent (rs1559200672)	DEE panel
EE87	chr2:g.166903479C>T	*SCN1A*	missense	c.1178G>A	Arg393His	LoF (PMID: 35037686)	het	not established**	LP (P if de novo)	recurrent (ClinVar ID: 68506; rs121917927)	DEE panel
EE35	chr2:g.166246152A>G	*SCN2A*	missense	c.5836A>G	Lys1946Glu	-	het	not established**	VUS (LP if de novo)	novel	DEE panel
EE64	chr2:g.166165725T>C	*SCN2A*	missense	c.656T>C	Phe219Ser	GoF (PMID: 35037686)	het	not established**	LP (P if de novo)	novel	DEE panel
EE125	chr2:g.166237708G>A	*SCN2A*	splice donor	c.4551+1G>A	-	LoF	het	not established**	LP	recurrent (rs527688117)	DEE panel
EE107	chr2:g.165969400C>T	*SCN3A*	missense	c.3838G>A	Val1280Ile	-	het	not established**	VUS (LP if de novo)	recurrent (rs751582800)	DEE panel
	arr[hg19] 5q35.2q35.3(176534633_177013961)x1	NA	CNV: loss	-	-	-	het	not established**	P	recurrent	CMA and MLPA
EE63	chr12:g.52099309G>A	*SCN8A*	missense	c.1243G>A	Glu415Lys	-	het	not established**	LP	recurrent (PMID: 35230384)	DEE panel
EE91	chr1:g.43408962C>T	*SLC2A1*	missense	c.49G>A	Gly17Arg	-	het	de novo	LP	recurrent (ClinVar ID 597357; rs1345986424)	DEE panel
EE99	chrX:g.53409426C>T	*SMC1A*	splice donor	c.3285+1G>A	-	LoF	het (female)	not established**	LP	recurrent (PMID: 31334757)	DEE panel
EE22	chr9:g.130435529C>T	*STXBP1*	stop gained	c.1099C>T	Arg367Ter	LoF	het	de novo	P	recurrent (ClinVar ID: 207429; rs796053366)	DEE panel
EIEE19	chr9:g.130444767G>C	*STXBP1*	missense	c.1630G>C	Gly544Arg	-	het	de novo	P	recurrent (ClinVar ID: 952587; rs1842044505)	DEE panel
EE126	chr9:g.130444788C>T	*STXBP1*	missense	c.1651C>T	Arg551Cys	-	het	not established**	P	recurrent (ClinVar ID: 207440; rs796053373)	DEE panel

Suppl. Table 5. cont. **Genetic Variants detected with the gene panel, ES and CMA (P/LP and VUS).**

pt.	genomic location (Hg19)	gene/s	variant type	coding DNA change (HGVS)	AA change	LoF/GoF*	zygosity	inheritance	ACMG classification	recurrent/ novel	Detection method
EE66	arr[hg19] 1p36.33p36.32(16637 01_5080691)x1	NA	CNV: loss	-	-	-	het	not established	P	recurrent	CMA and MLPA
EE104	arr[hg19] 4p16.3p16.1(71552_8 146008)x1	NA	CNV: loss	-	-	-	het	not established **	P	recurrent	CMA and MLPA
EE141	arr[hg19] 10q23.1(82932831_8 5857905)x1	*NRG3, NRG3-AS1, LOC105378397*	CNV: loss	-	-		het	not established **	VUS	non-recurrent	CMA
CNV284	arr[hg19] 12p13.33p11.1(173,7 86-34,835,836)x2-4 [0.02]	NA	CNV: mosaic gain	-	-	-	het	not established	P	non-recurrent	CMA (clinical lab)
EE89	arr[hg19] 13q13.3(35522735_3 6581416)x1	*NBEA, MAB21L1 , DCLK1, MIR54 8F5, LINC00445*	CNV: loss	-	-	-	het	not established **	LP	non-recurrent	CMA
EE42	arr[hg19] 14q12(29231406_292 42169)x1	*FOXG1, FOXG1-AS1, LINCO1551*	CNV: loss	-	-	-	het	not established **	LP	non-recurrent	CMA
EE48	15q11.2-q13 deletion	NA	CNV: loss	-	-	-	het	not established	P	recurrent	MLPA (private clinical lab)
EE65	arr[hg19] 16p13.11(14944560_ 16616189)x1	NA	CNV: loss	-	-	-	het	maternally	LP	recurrent	CMA and MLPA
EE103	arr[hg19] 22q11.21(18919942_ 20279820)x3	NA	CNV: gain	-	-	-	het	maternal	LP	recurrent	CMA and MLPA

NCBI RefSeq transcript and protein references: *ANGPT1*: NM_001146.3; NP_001137.2; *APC2*: NM_005883.2, NP_005874.1; *ATP1A2*: NM_000702.3, NP_872393.3; *CACNA1A*: NM_001127222.1, NP_001120694.1; *CELSR2*: NM_001408.2, NP_001399.1; *CHD2*: NM_001271.4, NP_001262.3; *CDKL5*: NM_003159.2, NP_003150.1; *COBILL1*: NM_001278461.1; *GLUL*: NM_001033044.3, NP_001028216.1; *KCNA2*: NM_004974.4, NP_004965.1; *KCNT1*: NM_020822.3, NP_065873.2; *NARS*: NM_004539.4, NP_004530.1; *PCDH19*: NM_001184880.2, NP_001171809.1; *PLPPR4*: NM_014839.4; *SCN1A*: NM_006920.4, NP_008851.3; *SCN2A*: NM_001040143.2, NP_001035233.1; *SCN3A*: NM_006922.4, NP_008853.3; *SCN8A*: NM_014191.4, NP_055006.1; *SLC2A1*: NM_006516.2, NP_006507.2; *SMC1A*: NM_006306.4, NP_006297.2; *STXBP1*: NM_003165.6, NP_003156.1; *UNC80*: NM_032504.2, NP_115893.1.

*LoF assumed for truncating variants

**DNA from both parents was unavailable for segregation analysis

***PMID: 30321769 - detected as part of published pilot

het: heterozygous; comp het: compound heterozygous

Suppl. Table 6. **Multiple Linear Regression Modelling to Assess Associations Between Selected Clinical Features and a Detected Candidate SNV/indel.**

Characteristic	**OR[1]**	**95% CI[1]**	**p-value**
	2.24	0.74, 6.46	0.14
Autism Age at 1st Seizure	0.98	0.95, 1.01	0.2
Movement Abnormality	6.08	2.49, 15.2	<0.001
Structural Brain Anomaly	1.82	0.36, 8.28	0.4
GDD	0.41	0.13, 1.12	0.10
Dysmorphism	1.89	0.53, 6.30	0.3
Attention Deficit	3.96	1.24, 12.3	0.018
[1]OR = Odds Ratio, CI = Confidence Interval			

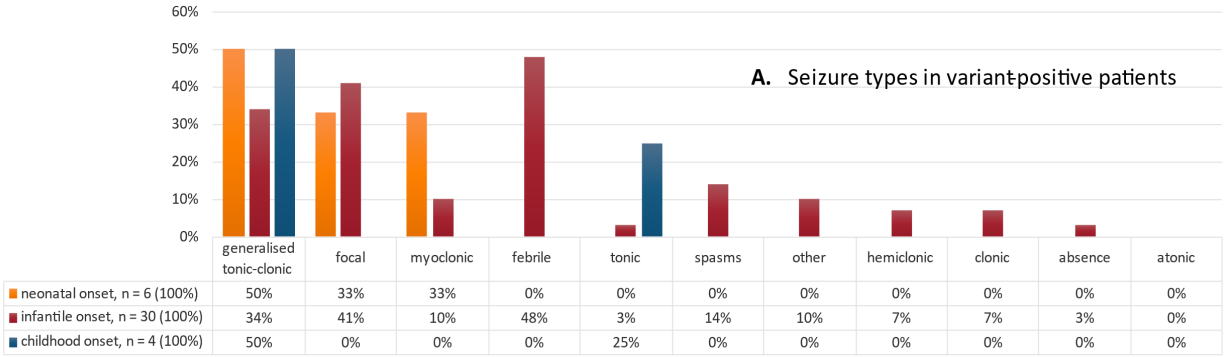
Suppl. Table 7. **Multiple Linear Regression Modelling to Assess Associations Between Selected Clinical Features and a Detected Candidate CNV.**

Characteristic	**OR[1]**	**95% CI[1]**	**p-value**
Autism	0.57	0.02, 4.92	0.7
Age at 1st Seizure	1.01	0.96, 1.05	0.5
Movement Abnormality	0.52	0.05, 3.27	0.5
Structural Brain Anomaly	22.8	3.25, 181	0.002
GDD	1.70	0.26, 9.91	0.6
Dysmorphism	4.52	0.63, 29.1	0.11
[1]OR = Odds Ratio, CI = Confidence Interval			

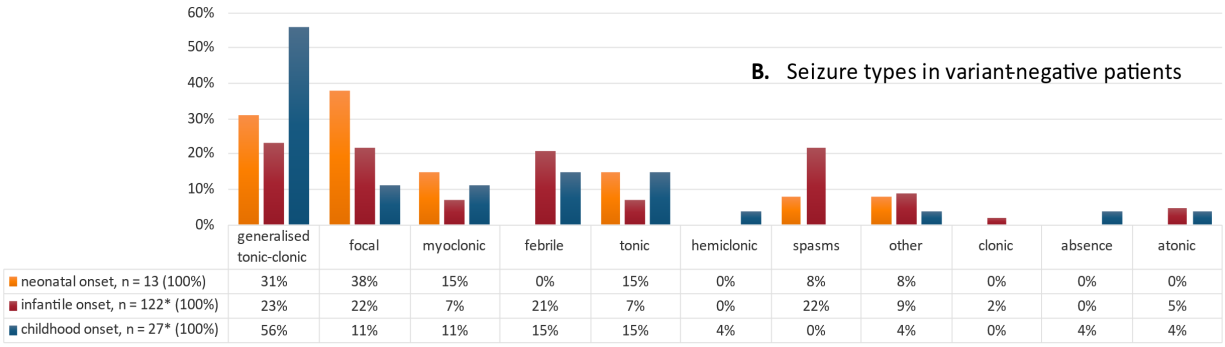
Suppl. Table 8. **Multiple Linear Regression Modelling to Assess Associations Between Selected Clinical Features and a Detected Candidate Genetic Variant (SNV/indel or CNV).**

Characteristic	**OR[1]**	**95% CI[1]**	**p-value**
Autism	2.11	0.72, 5.88	0.2
Age at 1st Seizure	0.99	0.96, 1.01	0.3
Movement Abnormality	5.00	2.12, 11.9	<0.001
Structural Brain Anomaly	10.6	2.14, 78.4	0.007
GDD	0.57	0.21, 1.43	0.3
Dysmorphism	3.13	0.98, 9.90	0.050
Attention Deficit	2.83	0.93, 8.32	0.060
[1]OR = Odds Ratio, CI = Confidence Interval			

6.2. Supplementary Figures

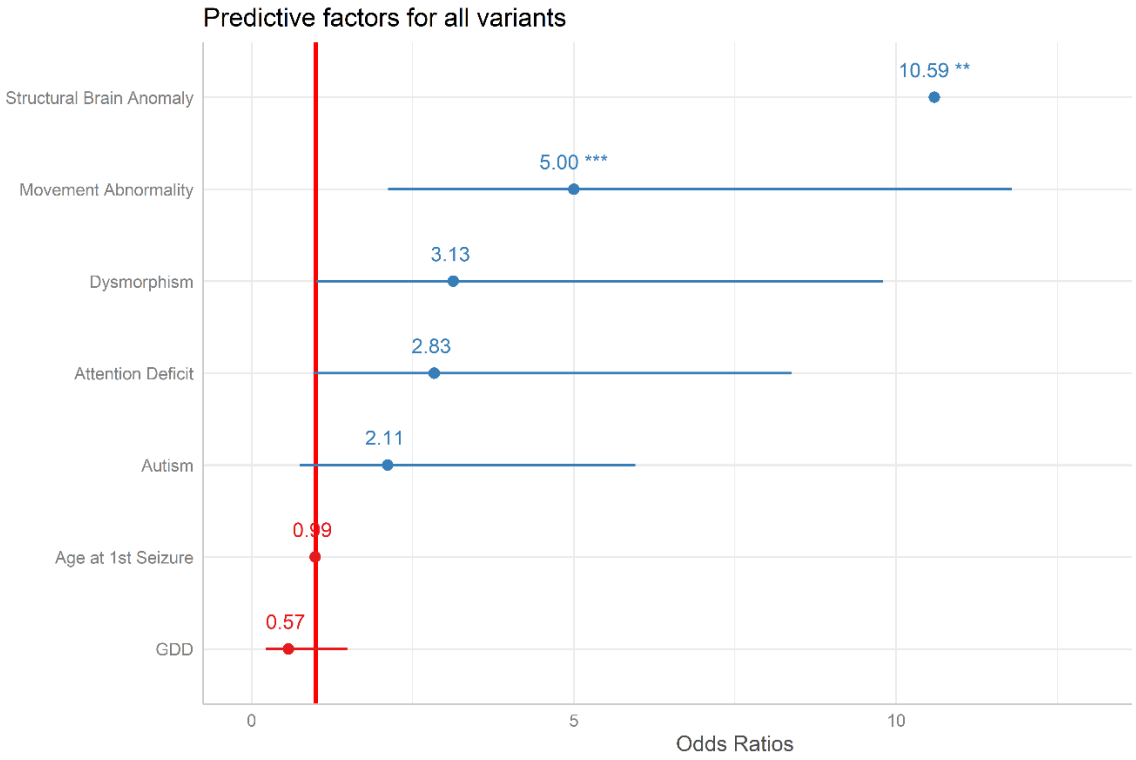


	generalised tonic-clonic	focal	myoclonic	febrile	tonic	spasms	other	hemiclonic	clonic	absence	atonic
neonatal onset, n = 6 (100%)	50%	33%	33%	0%	0%	0%	0%	0%	0%	0%	0%
infantile onset, n = 30 (100%)	34%	41%	10%	48%	3%	14%	10%	7%	7%	3%	0%
childhood onset, n = 4 (100%)	50%	0%	0%	0%	25%	0%	0%	0%	0%	0%	0%



	generalised tonic-clonic	focal	myoclonic	febrile	tonic	hemiclonic	spasms	other	clonic	absence	atonic
neonatal onset, n = 13 (100%)	31%	38%	15%	0%	15%	0%	8%	8%	0%	0%	0%
infantile onset, n = 122* (100%)	23%	22%	7%	21%	7%	0%	22%	9%	2%	0%	5%
childhood onset, n = 27* (100%)	56%	11%	11%	15%	15%	4%	0%	4%	0%	4%	4%

Suppl. Figure 1. **Seizure types in P/LP variant-positive (A) and P/LP variant-negative (B) children, stratified per age of seizure onset: neonatal, infantile and childhood.** *children with VUS were excluded.



Suppl. Figure 2: **Multiple Linear Regression modelling to assess associations between selected clinical features and presence of an identified SNV/indel or CNV (P/LP and VUS).**

6.3. Supplementary Notes

6.3.1. Suppl. Note 1: Essential Clinical Info Requested

DOB
Ethnicity
Neonatal complications
Age at 1st seizure
Age first seen
Initial seizure/s (type, frequency, special features e.g., hand movements)
Seizure evolution (type, frequency, special features e.g., hand movements)
Status epilepticus (number of events/duration)
Seizure triggers
ASMs trialled
Current ASMs
Ketogenic diet (trialled/ongoing/effect)
Response to treatment
History of non-adherence?
Development before seizure onset
Co-existing clinical features/conditions
Subsequent development
Seizure-associated neuroregression
Gait/movement abnormality
Other clinical features/conditions noted post seizure onset
Family history of seizures/epilepsy (details, if available)
EEG
Imaging: (type and findings)
Initial working diagnosis
Diagnostic genetic investigations and results
Final/current diagnosis

6.3.2. Suppl. Note 2: DEE MIP panel (71 genes):

Targeted NGS panel of 71 DEE-associated genes was performed using the previously described single molecule Molecular Inversion Probe (smMIP) technology (1). All exons and intron-exon boundaries (5-bp flanking sequences) were sequenced at 98% capture and 40X minimum depth of coverage (RefSeq, hg19 build)(2). NGS quality assessment, read alignment, depth of coverage, variant identification, annotation, prioritisation, and filtering was also performed using previously published methods (2–4). The VCF files were then subjected to further manual variant filtering and prioritisation. Genes included:

ALG13, ARX, ASH1L, ATP1A2, CACNA1A, CASK, CDKL5, CHD2, CLCN4, CUX2, DCX, DEPDC5, DMN1, DYRK1A, EEF1A2, FBXO28, FOXG1, FOXP1, GABRA1, GABRB1, GABRB2, GABRB3, GABRG2, GNAO1, GNB1, GRIN1, GRIN2A, GRIN2B, GRIN2D, HCN1, HCN2, HNRNPU, KCNA2, KCNB1, KCNH5, KCNQ2, KCNQ3. KCNT1, KIAA2022, MBD5, MECP2, MEF2C, NR2F1, PCDH19, PNKP, PNPO, PURA, RBFOX1, RORB, SCN1A, SCN1B, SCN2A, SCN3A, SCN8A, SIK1, SLC13A5, SLC1A2, SLC2A1, SLC35A2, SLC6A1, SMC1A, SNAP25, STX1B, STXBP1, SYN1, SYNGAP1, TBC1D24, TBL1XR1, TCF4, UBE3A, WDR45.

6.3.3. Suppl. Note 3: DEE-related genes with high coverage on the custom array (89 genes)

This targeted custom array incorporated high-coverage tiling over epilepsy-associated genes (n = 89), with one probe/150bp across the gene, 1 probe/300bp for 50kb flanking these genes, and 1 probe/500bp across recurrent microdeletions, as well as a backbone across the genome. The arrays were processed according to the manufacturer's instructions and analysed with the Agilent Cytogenomics v.5.1.2.1 Software using the Default Analysis Method - CGH v.2. CNVs were filtered to exclude regions that 1) did not include a coding gene/exon (Ref. build Hg19); 2) were smaller than 10 kb; or 3) had a 50% overlap with CNVs detected in healthy published controls (excluding the recurrent CNV regions 15q11.2, 16p13.11, 16p11.2)(5).

GNB1, MTOR, SLC2A1, KCNA2, CHRNB2, ASH1L, ATP1A2, CACNA1E, KCNH1, FBXO28, HNRNPU, LGI1, SLC25A22, KCNC1, SLC1A2, GRIN2B, SCN8A, FOXG1, DYNC1H1, UBE3A, GABRB3, CHRNA7, CHD2, NPRL3, TSC2, TBC1D24, RBFOX1, GRIN2A, STX1B, GNAO1, SLC13A5, PNPO, TCF4, HCN2, CACNA1A, SCN1B, GRIN2D, PNKP, MBD5, CACNB4, SCN3A, SCN2A, SCN1A, PLCB1, SNAP25, KCNB1, CHRNA4, KCNQ2, EEF1A2, DYRK1A, SIK1, DEPDC5, SLC6A1, NPRL2, FOXP1, TBL1XR1, GABRB1, HCN1, MEF2C, NR2F1, PURA, GABRB2, GABRA1, GABRG2, SYNGAP1, CHRNA2, KCNQ3, RORB, STXBP1, DNM1, TSC1, KCNT1, GRIN1, CLCN4, CDKL5, ARX, CASK, SYN1, SLC35A2, WDR45, IQSEC2, SMC1A, ARHGEF9, KIAA2022, BRWD3, PCDH19, DCX, MECP2, FLNA

6.3.4. Suppl. Note 4: DEE starter panel design (32 genes)

Underlined: genes associated with neonatal/infantile onset epilepsy syndromes, with actionable implications for treatment(6,7).

ALDH7A1, ARX, CACNA1A, CDKL5, CHD2, FOXG1, GABRA1, GABRG2, GRIN2A, GRIN2B, KCNA2, KCNB1, KCNQ2, KCNQ3, KCNT1, MECP2, PCDH19, PNPO, POLG, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, SCN9A, SLC25A22, SLC2A1, SLC6A1, STXBP1, SYNGAP1, TSC1, TSC2.

6.3.5. References for the Suppl. Information

1. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res [Internet]. 2013 May 1 [cited 2017 Aug 22];23(5):843–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23382536
2. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in *CHD2* and *SYNGAP1*. Nat Genet. 2013 Jul;45(7):825–30.
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5. MacDonald JR, Ziman R, Yuen RKC, Feuk L, Scherer SW. The Database of Genomic Variants: A curated collection of structural variation in the human genome. Nucleic Acids Res [Internet]. 2014 Jan 1 [cited 2021 Jun 16];42(D1). Available from: https://pubmed.ncbi.nlm.nih.gov/24174537/
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7. Bayat A, Bayat M, Rubboli G, Møller RS. Epilepsy Syndromes in the First Year of Life and Usefulness of Genetic Testing for Precision Therapy. 2021 Jul 8 [cited 2021 Nov 6];12(7):1051. Available from: https://www.mdpi.com/2073-4425/12/7/1051/htm

APPENDICES

APPENDIX 1: Ethical Approvals

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 April 2015

HREC REF: 232/2015

Prof R Ramesar
Human Genetics
Suite N3.17
Wernher & Beit North Building
IIDMM

Dear Prof Ramesar

PROJECT TITLE: DELINEATION OF THE GENETIC CAUSES OF EPILEPTIC ENCEPHALOPATHIES IN SOUTH AFRICAN INFANTS (PhD candidate - Ms A Esterhuizen)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 23 April 2015.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Ms Alina Esterhuizen will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

HREC 232/2015

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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Website: www.health.uct.ac.za/fhs/research/humanethics/forms

23 February 2016

HREC REF: 232/2015

Prof R Ramesar
Human Genetics
Clinical Lab Sciences
IIDMM

Dear Prof Ramesar

PROJECT TITLE: DELINEATION OF THE GENETIC CAUSES OF COMPLEX EPILEPSIES IN SOUTH AFRICAN PAEDIATRIC PATIENTS (PhD candidate - Ms A Esterhuizen)

Thank you for submitting your amendment to the Faculty of Health Sciences Human Research Ethics Committee dated 17 February 2016.

Thank you for the meeting as regards to the study and referral process and ability for the participants to obtain further analysis it needed.

It is a pleasure to inform you that the HREC has **noted and approved** the following amendments with reference to the above-mentioned study.

- Inclusion of Dr Birgit Schlegel as the study co-investigator.
- Change of title from "**delineation of the genetic causes of epileptic encephalopathies in South African infants**" to "**delineation of the genetic causes of complex epilepsies in South African paediatric patients**".
- An extension of the study cohort to include patients with early onset epileptic encephalopathies (EE) as well as those subsequently diagnosed with the tuberculosis sclerosis complex (TSC)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

pp TJB urges

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE
29 JUN 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30-5-23
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	30/6/2022

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website: http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	14/06/2022		
HREC REF Number	232/2015	Current Ethics Approval was granted until	30/05/2022
Protocol title	DELINEATION OF THE GENETIC CAUSES OF COMPLEX EPILEPSIES IN SOUTH AFRICAN PAEDIATRIC PATIENTS		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?		☐ Yes	☑ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	**Prof. R. Ramesar**		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Parental Consent Form

INFORMATION SHEET AND PARENTAL CONSENT FORM FOR PARTICIPATION IN A RESEARCH PROJECT

Study Investigators:
Prof. Raj Ramesar: Division of Human Genetics, Dept. of Pathology, Faculty of Health Sciences, UCT; Tel: 021 406 6995; e-mail: Raj.Ramesar@uct.ac.za
Prof. Jo Wilmshurst: Paediatric Neurology, Red Cross Children's Hospital; tel: 021 658 5111 ext 5434, e-mail: Jo.Wilmshurst@uct.ac.za
Dr. Gemma Carvill (PhD), Postdoctoral Research Fellow, Division of Genetic Medicine (DGM), University of Washington (UW), Seattle.
Mrs. Alina Esterhuizen: Div. of Human Genetics, Dept. of Pathology UCT/NHLS, tel: +27 21 406 6456, e-mail: Alina.Esterhuizen@uct.ac.za

Project title: **Delineation of the Genetic Causes of Complex Epilepsies in South African Paediatric Patients.**

PROJECT BACKGROUND

Epilepsy is a term given to a medical condition where the patient suffers from epileptic seizures ("fits"). It is one of the most common illnesses of the brain, and is most often seen in children, especially infants, who require special care. There are many different types and causes of epilepsy and many of them are serious and difficult to treat. Often, in addition to seizures, the child suffers from learning problems and delayed motor development. These get worse, if the correct treatment is not available early on. However, epilepsy disorders are complicated and really difficult to diagnose properly, because the signs and symptoms are varied and change with the growth of the child. The aim of this study is to gain knowledge which may be useful in the diagnosis of the complex epilepsies, and may translate into improved diagnosis and therapies in the future.

Scientists have shown that many types if epilepsies are caused by genetic mutations. Genes are made up of DNA, which is a long and complex molecule that carries the code for every substance and function in the human body. Genes and DNA are passed on (or inherited) in a specific way from parents to their children. Occasionally, the DNA code carries "a mistake", known as a mutation, which can cause illness. A disease-causing mutation can either be passed on from a parent to the child (in other words, it is inherited) or it can originate in the child (*de novo*). Recognising a genetic illness (one caused by DNA mutations) is easy if it can be seen to "run" in the family. Often however, epilepsy is not seen to "run" in families. Rather, the mutations arise new (*de novo*) in the child's DNA. For this reason, the role of genetics in epilepsy was not fully recognized in the past.

Genetic testing has been used for many years to confirm or exclude a number of diseases. Because epilepsy is genetically complicated, testing is often difficult and expensive. However, a newly available sophisticated medical technology is making genetic testing for epilepsy available in many parts of the developed world (USA, UK and Europe). This research study will use this new technology (next generation sequencing - NGS) to study the genetic causes of epilepsies in South African children, ultimately hoping to develop genetic tests for proper and quicker diagnosis of children with epilepsy.

SELECTION AND RECRUITMENT

Blood Samples

We request that **blood specimens from you (both parents, if possible) and your child** be donated to this project. If possible, 2 x 2 - 5ml of blood will be collected into EDTA tubes (purple top), and used to extract DNA for genetic analysis. Your child, who is suffering from epilepsy, will be the primary subject of this project. However, since this is a genetic study, your DNA may be needed to confirm the disease-association of any mutation detected in your child. This does not mean that you may develop epilepsy like your child. Rather, it will help the researchers to decide with more certainty, if a genetic or mutation is the cause of epilepsy in your child.

What will happen to our DNA samples?

This research project is conducted in collaboration with a genetics research centre at the University of Washington USA, where some of the testing will take place. All the DNA samples will then be returned to South Africa, and after completion of the study, will be **stored in the DNA Registry of the Division of Human Genetics (HREC REF 217/2010)** under a specific DNA code, for an unspecified period of time. The DNA is stored in case further genetic studies become available in the research study area of EE. You will be contacted and your permission gained, if additional studies are thought to be of possible relevance to your child.

Will I be informed of the results?

Yes. Your doctor will inform you if and what type of an epilepsy-causing mutation was found in your child, and what it means in terms of his/her diagnosis and treatment. You will also be told if no mutation was found. However, it is important remember that research projects are conducted over long periods of time (months or years), so this information will not be available for a long time after sampling.

If, during this research, your child's a mutation is also found in your DNA, the meaning and implications of this finding will be explained to you in detail by your child's doctor. As part of the clinical service, you will be given an option to discuss your results with a genetic counsellor. Further intervention/referral or testing will be offered to you, if required.

Voluntary Participation and Confidentiality

- Participation as a subject or control (in this case, the parent) is completely voluntary.
- You reserve the right to refuse to participate in the study.
- A decision to refuse will not affect your child's treatment now or in the future.
- You are free to withdraw consent at any time.
- All information given will remain strictly confidential. Each specimen will be stored in a secure location and assigned an anonymous code known only to the investigators working on the project.
- You may request to have your child's and/or sample/s removed from the Repository and discarded after the completion of the study.
- This study is being conducted according to the principles of the Declaration of Helsinki (Brazil 2013), which looks after the interest of the participants.

Parental Consent Form cont.

WHAT IF SOMETHING GOES WRONG?

The University of Cape Town (UCT) undertakes that in the event of your child suffering any significant deterioration in health or well-being, or from any unexpected sensitivity or toxicity, that is caused by his participation in the study, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial-related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly-speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

Participants may contact the Human Research Ethics Committee at the UCT with any questions or concerns about their rights or welfare as research participants: tel: +27 21 406 6338; fax: +27 21 406 6411; Email: nosi.tsama@uct.ac.za or shuretta.thomas@uct.ac.za

Benefits of the Study

The study offers no direct benefit to the participants or their families. The research is however conducted with the long term view of establishing a diagnostic genetic service for epilepsy. Participation may therefore have a long term benefit in helping to develop and improve the diagnosis and treatment of epilepsy in South Africa.

This research protocol has been approved by the Department of Clinical Laboratory Sciences Research Committee as well as the Research Ethics Committee of the Faculty of Health Sciences of UCT (HREC REF 232/2015).

If you have further questions, you can contact:

Prof. Jo Wilmshurst: Paediatric Neurology, Red Cross Children's Hospital; tel: 021 658 5111 ext 5434, e-mail: Jo.Wilmshurst@uct.ac.za

Mrs. Alina Esterhuizen: Div. of Human Genetics UCT/NHLS, tel: +27 21 404 4550/406 6456, e-mail: Alina.Esterhuizen@uct.ac.za

Project title: **Delineation of the Genetic Causes of Complex Epilepsies in South African Paediatric Patients.**

PARENTAL CONSENT

PLEASE TICK WHERE RELEVANT:

1. I agree to the sampling and use of my child's blood in this study. ☐ Yes ☐ No
   I agree to the sampling and use of my blood in this study. ☐ Yes ☐ No
2. I have been informed that my child's blood sample and DNA will be assigned a unique identification code. This code and the link between the code and my child's name will be known only to the investigators in the study. ☐ Yes ☐ No

   I have been informed that my blood sample and DNA will be assigned a unique identification code. This code and the link between the code and my name will be known only to the investigators in the study. ☐ Yes ☐ No
3. I understand that a part of this research project will be conducted in the USA and I give permission for my child's DNA to be transported and tested there. ☐ Yes ☐ No

   I understand that a part of this research project will be conducted in the USA and I give permission for my DNA to be transported and tested there. ☐ Yes ☐ No
4. I agree to the use of my child's DNA in future research project/s approved by the Research Committee of the Faculty of Health Sciences at UCT. ☐ Yes ☐ No

   I agree to the use of my DNA in future research project/s approved by the Research Committee of the Faculty of Health Sciences at UCT. ☐ Yes ☐ No
5. I agree to the storage of my child's DNA ☐ Yes ☐ No
   I agree to the storage of my DNA ☐ Yes ☐ No
6. I understand that the results of this project may be used as theses material for higher degrees, and publication in scientific journal/s. ☐ Yes ☐ No
7. I understand that the results of this research project may provide information about the genetic background of my child's illness. These results will not provide information about my child's complete genetic makeup. ☐ Yes ☐ No
8. ALL OF THE ABOVE HAS BEEN EXPLAINED TO ME IN A LANGUAGE THAT I UNDERSTAND AND MY QUESTIONS ANSWERED BY:

   _____

Child's Name (print)_____

Mother's Name (print)_____Signature_____

Father's name (print)_____Signature_____

Legal Guardian's Signature (if other than biological parents): _____

Date: _____

This research protocol has been approved by the Department of Clinical Laboratory Sciences Research Committee as well as the Research Ethics Committee of the Faculty of Health Sciences of UCT (HREC REF: 232/2015).

Assent Form

INFORMATION SHEET AND ASSENT FORM FOR PARTICIPATION IN A RESEARCH PROJECT

Study Investigators:
Prof. Raj Ramesar: Division of Human Genetics, Dept. of Clinical Laboratory Sciences, Faculty of Health Sciences, UCT; Tel: 021 406 6995; e-mail: Raj.Ramesar@uct.ac.za
Prof. Jo Wilmshurst: Paediatric Neurology, Red Cross Children's Hospital; tel: 021 658 5111 ext 5434, e-mail: Jo.Wilmshurst@uct.ac.za
Dr. Gemma Carvill (PhD), Postdoctoral Research Fellow, Division of Genetic Medicine (DGM), University of Washington (UW), Seattle.
Mrs. Alina Esterhuizen: Div. of Human Genetics UCT/NHLS, tel: +27 21 404 4550, e-mail: Alina.Esterhuizen@uct.ac.za

Project Title: **Delineation of the Genetic Causes of Complex Epilepsies in South African Paediatric Patients.**

Why we would like to speak with you

We want to talk with you about being a part of something called a research study. A research study is when doctors and scientists collect information to learn more about a disease. If you have any questions during our talk about this study, please ask - you can ask now or later.

We are doing this research study to learn more about children with epilepsy. After we tell you about it, we will ask you if you'd like to take part in this research, or not. If you decide that you'd like to take part, you will be asked to sign this paper and you can take a copy of it home with you. It's okay to say 'NO' if you don't want to be in the study. Even if you say 'YES', you can change your mind and say 'NO' later. You are welcome to speak with your parent/guardian about your decision.

Why are we doing this research study?

It can be tricky for the doctors to know for sure what kind of epilepsy a child has. That is why it can take a long time and many visits to the doctor, before some children get the right kind of medicine. We want to do this research to find a test that will give the right answer straight away and help the doctors decide what medicine and treatment should work best.

What will happen to you if you are in the research study?

Your doctor has already explained to you about taking a blood sample into a purple topped tube (about 3ml - a tablespoonful) to help us find out why you have epilepsy. If you say 'NO' to being in the study, we will not take any samples from you (though your blood may be drawn for other lab tests).

If you say 'YES' we will test your sample in the laboratory and look for the reason you have epilepsy. We will be working together with other scientists overseas and some of the testing will be done in America. This means that your sample may be taken to America for testing - but it will come back afterwards and stay here at the University of Cape Town.

In case you're wondering, we will be asking other children with the same illness to be in this research study. Your illness is quite special, so there will not be very many children we can ask.

Will it hurt?

This blood sample will be drawn in the same way as blood samples taken from you before, for other laboratory tests. You are going to feel a small prick of a needle and it will be over quickly. The doctors and nurses are very good at this and will explain to you exactly what they are going to do. It is very important to tell your parents and the doctors if you feel scared or if anything hurts.

Do you have to be in this research study?

Your parents have said that it is okay for you to be a part of the study – but you can still say 'No'. No-one will be upset with you if you don't want to take part. And, remember, you can say yes now and change your mind later. It's up to you.

What if you have questions?

You may ask questions at any time. You can ask now or later. You may talk to the doctor or someone else. We have given your parents/guardians the contact numbers of the persons you, or they, can call after you go home.

This research protocol has been approved by the Department of Clinical Laboratory Sciences Research Committee as well as the Research Ethics Committee of the Faculty of Health Sciences of UCT (HREC REF: 232/2015).

Project Title: **Delineation of the Genetic Causes of Complex Epilepsies in South African Paediatric Patients.**

Saying Yes or No to being in this research study

You can say Yes or No. If you say Yes, remember:

- You can stop being in the study any time you want to.
- You can call the doctor any time you have any questions.
- Besides your parents/guardians, your information will only be shared with the doctors and scientists in this study.

Please put X next to your answer:
Can we take your blood sample to test in our research project?

YES____NO____

If you have signed this paper, it means you have had all your questions for today answered and you want to be in the research study.

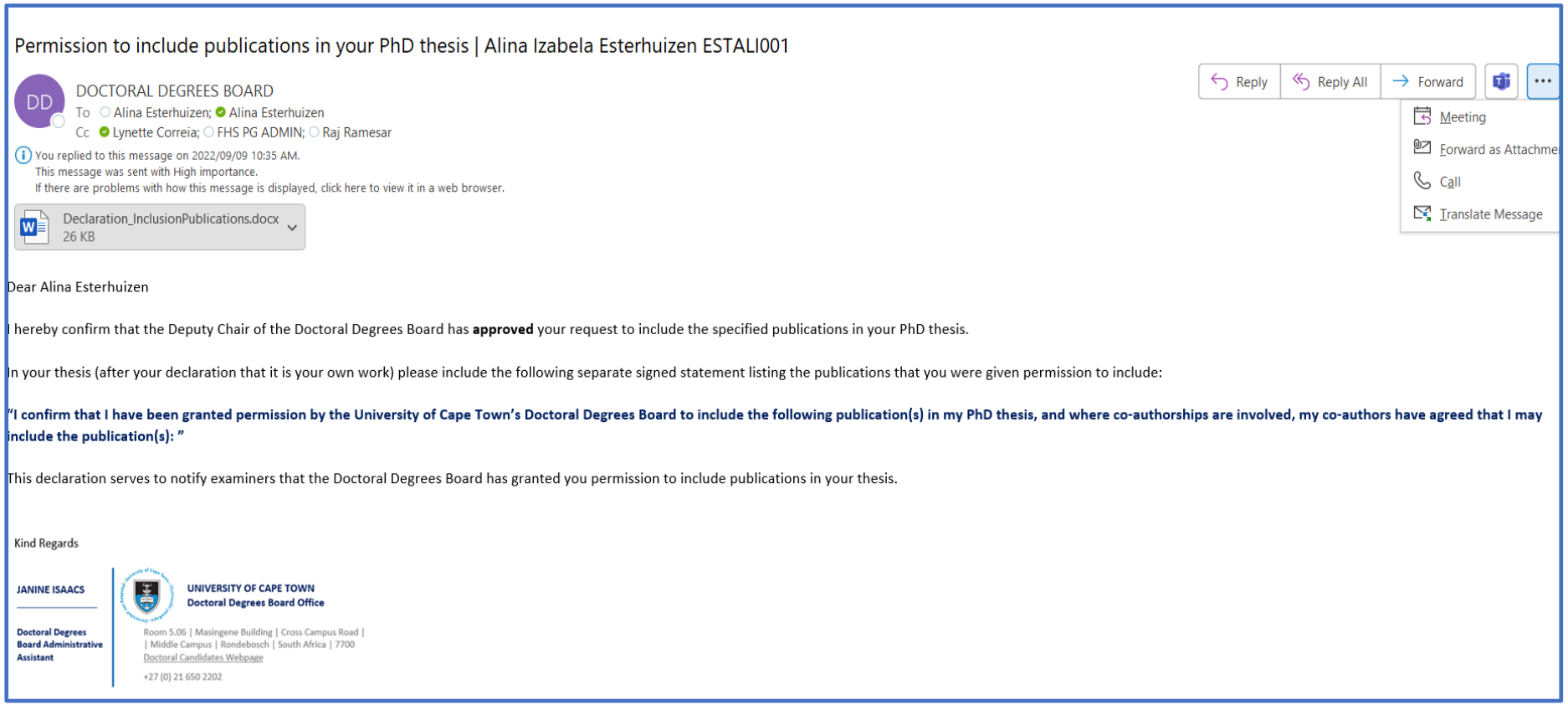
If you don't want to be in the study, don't sign this paper.
Being in the study is up to you, and no one will be upset if you don't sign this paper or if you change your mind later.

_____
Signature of Child *Date*

_____
Signature of Person Obtaining Assent/Consent *Date*

This research protocol has been approved by the Department of Clinical Laboratory Sciences Research Committee as well as the Research Ethics Committee of the Faculty of Health Sciences of UCT (HREC REF: 232/2015).

APPENDIX 2: Permission to include publications



Permission to include publications in your PhD thesis | Alina Izabela Esterhuizen ESTALI001

DOCTORAL DEGREES BOARD
To Alina Esterhuizen; Alina Esterhuizen
Cc Lynette Correia; FHS PG ADMIN; Raj Ramesar

Reply | Reply All | Forward | Meeting | Forward as Attachme | Call | Translate Message

You replied to this message on 2022/09/09 10:35 AM.
This message was sent with High importance.
If there are problems with how this message is displayed, click here to view it in a web browser.

Declaration_InclusionPublications.docx
26 KB

Dear Alina Esterhuizen

I hereby confirm that the Deputy Chair of the Doctoral Degrees Board has **approved** your request to include the specified publications in your PhD thesis.

In your thesis (after your declaration that it is your own work) please include the following separate signed statement listing the publications that you were given permission to include:

"I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s): "

This declaration serves to notify examiners that the Doctoral Degrees Board has granted you permission to include publications in your thesis.

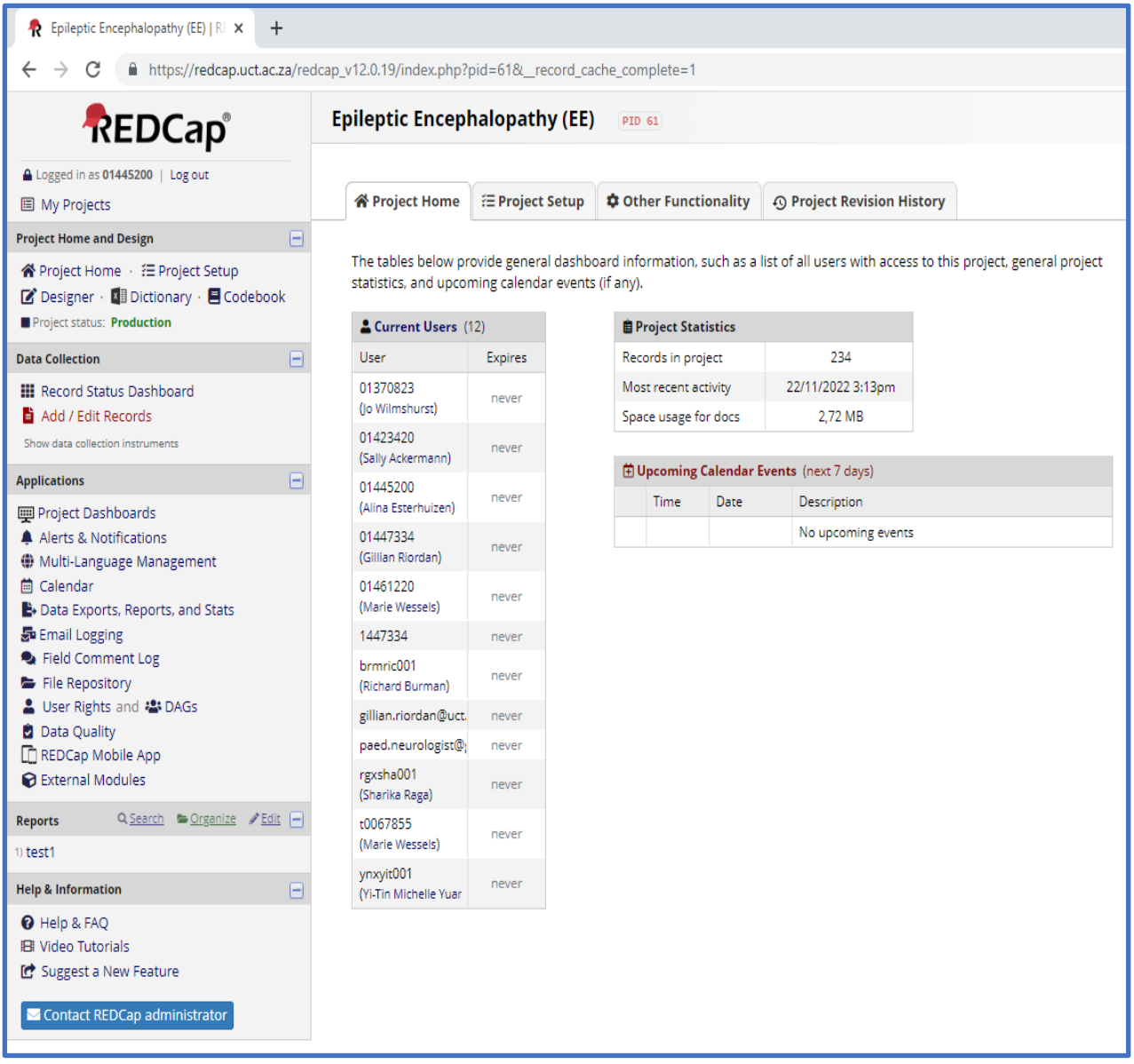
Kind Regards

JANINE ISAACS
Doctoral Degrees Board Administrative Assistant

UNIVERSITY OF CAPE TOWN
Doctoral Degrees Board Office
Room 5.06 | Masingene Building | Cross Campus Road | | Middle Campus | Rondebosch | South Africa | 7700
Doctoral Candidates Webpage
+27 (0) 21 650 2202

Appendix 3: REDCap database

Home page:



REDCap instruments: