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Epilepsy in the mTORopathies: molecular mechanisms and precision medicine opportunities

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Supervisor(s): Professor N Delanty and Professor G Cavalleri

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Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of higher degree of Doctor of Medicine is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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A

List of abbreviations

AAV= adeno-associated virus

ACGS= Association of Clinical Genomic Science

ACMG= American College of Medical Genetics and Genomics

ACTH= adrenocorticotropic hormone

AD= autosomal dominant

AML= angiomyolipoma

AMPK= adenosine monophosphate-activated kinase

ASD= autism spectrum disorder

ASM= anti-seizure medication

ASO= antisense oligonucleotide

ATP= adenosine triphosphate

BOSD= bottom-of-sulcus dysplasia

bp= base pair

CBD= cannabidiol

CCM= cerebral cavernous malformation

CHI= Children's Health Ireland

CGH= comparative genomic hybridisation

CGI-I= Clinical Global Impression of Improvement

CNS= central nervous system

CNV= copy number variant

COVID-19= Coronavirus disease 2019

CRISPR= clustered regularly interspaced short palindromic repeats

CSF= cerebrospinal fluid

CYP3A4= cytochrome P450 3A4

C12orf66= chromosome 12 open reading frame 66

DALY= disability-adjusted life-years

DBS= deep brain stimulation

DEE= developmental and epileptic encephalopathy

DEPDC5= Dishevelled, Egl-10 and Pleckstrin domain-containing protein 5

DNA= deoxyribonucleic acid

DNET= dysembryoplastic neuroepithelial tumour

DRE= drug-resistant epilepsy

ECG= electrocardiograph

EEG= electroencephalogram

EMA= European Medicines Agency

EPR= Electronic Patient Record

ESRM= epilepsy surgery review meeting

FCD= focal cortical dysplasia

FDA= Food and Drug Administration

FDG= fluorodeoxyglucose

FFEVF= familial focal epilepsy with variable foci

FKBP12= FK506 binding protein 1 A 12 kDa

GABA= γ-aminobutyric acid

GAMT= guanidinoacetate methyltransferase

GAP= GTPase-activating protein

GATOR1= GAP activity towards Rags 1

GEFS+= generalised epilepsy with febrile seizures plus

GLUT1= glucose transporter 1

GoF= gain-of-function

gnomAD= The Genome Aggregation Database

GWAS= genome wide association studies

HLA= human leukocyte antigen

HME= hemimegalencephaly

HRCDC= Health Research Consent Declaration Committee

ID= intellectual disability

IGE= idiopathic generalised epilepsies

ILAE= International League Against Epilepsy

indel= insertion/deletion

iPSC= induced pluripotent stem cell

IQR= interquartile range

ITFG2= integrin alpha FG-GAP repeat containing 2

KICSTOR= KPTN, ITFG2, C12orf66 and SZT2-containing regulator of mTORC1

KPTN= kaptin

LAM= lymphangioleiomyomatosis

LKB= liver kinase B

LoF= loss-of-function

MAF= minor allele frequency

MCAP= megalencephaly capillary malformation-polymicrogyria syndrome

MCD= malformation of cortical development

MDT= multidisciplinary team

MLPA= multiplex ligation probe amplification

MMSF= mean monthly seizure frequency

MOGHE= mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy

MPPH= megalencephaly polydactyly polymicrogyria-hydrocephalus syndrome

MRI= magnetic resonance imaging

mRNA= messenger ribonucleic acid

mTOR= mechanistic target of rapamycin

mTORC1= mTOR complex 1

mTORC2= mTOR complex 2

MTS= mesial temporal sclerosis

NF1= neurofibromatosis type 1

NGS= next-generation sequencing

NPRL2= nitrogen permease regulator-like 2

NPRL3= nitrogen permease regulator-like 3

NMD= nonsense-mediated mRNA decay

OMIM= Online Mendelian Inheritance in Man

PCR= polymerase chain reaction

PDK1= phosphoinositide-dependent kinase 1

PET= positron emission tomography

PI3K= phosphoinositide 3-kinase

PKB= protein kinase B

PMSE= polyhydramnios, megalencephaly and symptomatic epilepsy

PNES= psychogenic non-epileptic seizures

PRS= polygenic risk score

PTEN= phosphatase and tensin homologue

PVNH= periventricular nodular heterotopia

PWE= people with epilepsy

RABGAP1= Rab GTPase-activating protein 1

RCSI= Royal College of Surgeons in Ireland

Rheb= Ras homologue enriched in brain

RNA= ribonucleic acid

RNS= responsive neurostimulation

SARS-CoV-2= Severe Acute Respiratory Syndrome Coronavirus 2

SE= status epilepticus

SEEG= stereoelectroencephalography

SEGA= subependymal giant cell astrocytoma

SPECT= single-photon emission computed tomography

STRADα= STE20-related kinase adaptor alpha

SUDEP= sudden unexpected death in epilepsy

SWI= susceptibility-weighted imaging

SZT2= seizure threshold 2

S6K= ribosome S6 kinase

TAND= tuberous sclerosis complex-associated neuropsychiatric disorders

TANGO= targeted augmentation of nuclear gene output

TBC1D7= Tre2-Bub2-Cdc16 1 domain family member 7

TFE3= transcription factor E3

TOR= target of rapamycin

TOSCA= tuberous sclerosis registry to increase disease awareness

TSC= tuberous sclerosis complex

VAF= variant allele frequency

vEEG= video electroencephalography

VNS= vagus nerve stimulation

VUS= variant of uncertain significance

WES= whole exome sequencing

WGS= whole genome sequencing

C

Summary

Advances in next-generation sequencing and bioinformatics have accelerated gene discovery in severe epilepsies, with a specific genetic cause identified in up to 40% of epileptic encephalopathies. Precision medicine strives to tailor treatment to individual genetic characteristics and biology. The mechanistic target of rapamycin (mTOR) signalling cascade serves as a ubiquitous regulator of cell growth and metabolism, in response to growth factors, nutrients and energy. Pathogenic variation in genes encoding components of the mTOR cascade cause epilepsies and neuropsychiatric disorders, through hyperactivated mTOR signalling. Tuberous sclerosis complex (TSC) is characterised by multisystem benign tumours and refractory epilepsy, caused by inactivating variants in mTOR regulators *TSC1* or *TSC2*. Pathogenic variants in *DEPDC5*, *NPRL2* and *NPRL3* encoding the GAP activity towards Rags 1 (GATOR1) complex, primarily cause non-lesional focal epilepsies, as well as focal cortical dysplasia. GATOR1 inhibits mTOR activity in response to cellular amino acid levels. In the first large-scale precision medicine trial for a monogenic epilepsy, treatment with everolimus (a synthetic mTOR inhibitor) improved seizure control in people with TSC.

The primary objective of this thesis is to describe and evaluate the application of precision medicine to the management of epilepsy in mTORopathies, with a specific focus on GATOR1-related epilepsies. This thesis comprises three literature-based studies that review: (a) the clinical and genetic spectrum of mTORopathies, (b) evidence supporting mTOR inhibitor use in non-TSC mTORopathies, and (c) epilepsy surgery outcomes in patients with monogenic epilepsies. The primary investigations consist of: (a) a retrospective study of everolimus in a cohort of TSC patients with refractory epilepsy, (b) a deep phenotyping analysis of patients with GATOR1-related epilepsies, and (c) a prospective study of everolimus treatment for refractory GATOR1-related epilepsies. This work provides the first clinical data on the potential benefit of everolimus precision therapy for refractory epilepsies caused by loss-of-function variants in *DEPDC5.* The findings suggest that everolimus may be a viable treatment option for *DEPDC5* mTORopathy when considered in conjunction with established high-efficacy treatments such as epilepsy surgery. Larger studies are still required to support this recommendation.

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Publications and presentations

Publications arising from the primary work of this project

- **Moloney PB**, Cavalleri GL and Delanty N. Epilepsy in the mTORopathies: opportunities for precision medicine. *Brain Commun*. 2021 Sep 25; 3(4): fcab222.
- **Moloney PB**, Dugan P, Widdess-Walsh P, Devinsky O and Delanty N. Genomics in the presurgical epilepsy evaluation. *Epilepsy Res.* 2022 Aug; 184: 106951.
- **Moloney PB** and Delanty N. Stick or twist: Everolimus for seizures in tuberous sclerosis complex during the COVID-19 pandemic. *Seizure*. 2021 Oct; 91: 271-272.
- Behan C, Davis R, Vasseghi M, **Moloney PB**, Amin S, Delanty N and Doherty CP. Tuberous Sclerosis: A Rare Disease with an Orphan Complex. *Ir Med J*. 2022 Aug 18; 115(7): 635.
- **Moloney PB**, Kearney H, Benson KA, Costello DJ, Cavalleri GL, Gorman KM, Lynch BJ and Delanty N. Everolimus precision therapy for the GATOR1-related epilepsies: a case series. *Under review*.

Selected presentations arising from this project

- **Moloney PB**, Kearney H, Cavalleri GL and Delanty N. Targeted therapy for *DEPDC5* mTORopathy. *Irish Chapter of ILAE 10th Annual Expert Day.* Virtual; April 2021 [oral presentation].
- **Moloney PB**, Behan C, Doherty CP, Costello DJ, El-Naggar H, Widdess-Walsh P and Delanty N. Everolimus for drug-resistant seizures in tuberous sclerosis complex: an Irish experience. *Irish Neurology Association Annual Meeting*. Virtual; May 2021 [oral presentation].
- **Moloney PB**, Doyle M, Kearney H, El-Naggar H, Benson KA, Cavalleri GL and Delanty N. Everolimus as a precision therapy for drug-resistant epilepsy caused by mutations in the GATOR1 complex genes *DEPDC5* and *NPRL3*. *The 34th*

ILAE International Epilepsy Congress. Virtual; September 2021 [oral presentation].

- **Moloney PB**, Doyle M, Kearney H, White M, Behan C, Doherty CP, El-Naggar, Widdess-Walsh P, Benson KA, Cavalleri GL and Delanty N. Epilepsy in the mTORopathies: opportunities for precision medicine. *Irish Registrars' Prize in Clinical Neuroscience.* Virtual; November 2021 [oral presentation].
- **Moloney PB**, Doyle M, Kearney H, Benson KA, El-Naggar H, Costello DJ, Cavalleri GL and Delanty N. Everolimus as a Precision Therapy for Drug-Resistant Seizures in the GATORopathies. *American Epilepsy Society Annual Meeting*. Chicago; December 2021 [poster presentation].
- **Moloney PB**, Kearney H, Doyle M, White M, Greally M, Costello DJ, Lynch B, Gorman K, Benson KA, Cavalleri GL and Delanty N. Everolimus targeted therapy for *DEPDC5* mTORopathy. 5th *Dianalund International Conference on Epilepsy (DICE)*. Denmark; April 2022 [oral presentation]. ***Awarded travel bursary****.*
- **Moloney PB**, Kearney H, Doyle M, White M, Greally M, Costello DJ, Lynch B, Gorman K, Benson KA, Cavalleri GL and Delanty N. Everolimus targeted therapy for *DEPDC5* mTORopathy. *Irish Neurology Association Annual Meeting*. Kilkenny; May 2022 [poster presentation]. ***Awarded the Dr. John Lynch Prize for best poster****.*
- **Moloney PB**, Kearney H, Benson KA, Costello DJ, Cavalleri GL, Gorman KM, Lynch BJ and Delanty N. Initial experience using everolimus targeted therapy in the GATOR1 complex epilepsies – a case series. *ILAE British Branch Annual Meeting*. Cardiff; October 2022 [oral presentation]. ***Awarded travel bursary****.*
- **Moloney PB**. Genetics of Focal Epilepsy. *The 21st Annual Neurology Update Meeting*. Dublin; October 2022 [oral presentation]. **Invited speaker**.

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Name	Title	Affiliation
Prof. Bryan Lynch	Consultant Paediatric Neurologist	Children's Health Ireland at Temple Street
Dr. Kathleen Gorman	Consultant Paediatric Neurologist	Children's Health Ireland at Temple Street
Prof. Daniel Costello	Consultant Neurologist	Cork University Hospital
Prof. Colin Doherty	Consultant Neurologist	St. James's Hospital
Ms. Claire Behan	Epilepsy Specialist Nurse	St. James's Hospital
Dr. Peter Widdess-Walsh	Consultant Neurologist	Beaumont Hospital
Dr. Hanny El-Naggar	Consultant Neurologist	Beaumont Hospital
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1. Introduction

The goal of this thesis is to examine the potential of applying a precision medicine approach to the management of epilepsy in mTORopathies. Treatment strategies in the epilepsies have shifted from a "*one-size-fits-all*" approach to a more precise therapeutic paradigm, with anti-seizure medications (ASM) now targeted to seizure types (for example, rufinamide for tonic and atonic seizures), epilepsy syndromes (for example, cannabidiol for Lennox-Gastaut syndrome) and genetic aetiologies (for example, ganaxolone for *CDKL5* deficiency disorder)[1]. The terms precision, personalised and stratified medicine are often used interchangeably, despite having distinct meanings in the literature (see **Table 1.1**)[2]. For this thesis, the term "*precision medicine*" describes treatment strategies targeted at the underlying disease biology, most commonly molecular-genetic factors[1].

Many clinicians contend that they have always practiced stratified and personalised medicine. "*Stratified medicine*" utilises biomarkers or diagnostic tests to identify patient subgroups with differences in disease susceptibility and prognosis, and to predict response to treatment[3]. The most successful application of stratified medicine within epilepsy has been the use of human leukocyte antigen (HLA) genotyping, to identify at-risk populations for serious adverse events with carbamazepine and phenytoin treatment. In patients exposed to carbamazepine and to a lesser extent phenytoin, the *HLA-B*1502* polymorphism (common in Han Chinese, Thai, Indian and Malaysian populations) is associated with an increased risk of Stevens-Johnson syndrome and toxic epidermal necrolysis syndrome[4, 5]. The U.S. Food and Drug Administration (FDA) and the U.K. Medicines and Healthcare Products Regulatory Agency (MHRA) recommend screening for the *B*1502* polymorphism in patients with Han Chinese ancestry prior to starting carbamazepine treatment. A second pharmacogenomic marker, *HLA-A*3101,* predisposes to less severe carbamazepine-induced cutaneous reactions[6, 7]. Regulatory agencies do not recommend screening of *A*3101* before starting carbamazepine, as hypersensitivity reactions are less severe and less common compared to patients with the *B*1502* allele. However, reduced costs of genetic testing and wider availability of broad pharmacogenomic panels, will likely lead

to increased application in neurology practice to stratify risk of adverse events across a range of therapies.

"*Personalised medicine*" can be considered as an extension of stratified medicine, where optimal therapeutic choices are considered at an individual level, as opposed to population subgroups. Personalised medicine accounts for the heterogeneity of individual patients by targeting treatment to distinguishing phenotypic, genetic and psychosocial characteristics[2]. Most physicians incorporate a personalised medicine approach to their everyday practice. Epilepsy clinicians often avoid levetiracetam as a first line treatment in patients with psychiatric co-morbidities, as psychiatric adverse events are common with this drug. In patients with poor ASM adherence, clinicians may choose once-daily treatments like eslicarbazepine over twice-daily treatments. These personalised therapeutic decisions improve patient outcomes by offering a patient centred approach. However, they are often based on the clinician's own experience and may not strictly represent evidence-based medicine. For this reason, the term "*precision medicine*" is distinct to personalised medicine, reserved for the description of treatments that target underlying biological mechanisms.

There are a few notable examples of treatments that address the primary biological mechanisms underlying specific genetic epilepsies. For example, the ketogenic diet is an effective epilepsy treatment in glucose transporter 1 (GLUT1) deficiency syndrome caused by pathogenic variants in the *SLC2A1* gene[8]. Further examples of precision treatments will be addressed in greater detail in later sections. This exploration takes place on the backdrop of recent major advances in next-generation sequencing (NGS) technology and associated bioinformatics. Precision diagnostics have accelerated gene discovery in the epilepsies, with a specific genetic cause identified in up to 40% of patients with severe epilepsy syndromes and co-morbid intellectual disability (ID)[9, 10].

This thesis involves three literature-based studies and three primary investigations. Findings from these studies have been used to better understand the potential of a precision medicine approach in a well-defined group of genetic epilepsies. The introduction will provide a general background on several key conceptual issues that

recur throughout the thesis, including classifying epilepsies according to aetiology, drug-resistance, monogenic epilepsies and precision medicine.

Table 1.1 Definitions of stratified, personalised and precision medicine and examples of applications in the management of epilepsy

Term	Definition	Epilepsy examples
Stratified medicine	Using biomarkers or diagnostic tests to identify patient subgroups with differences in disease susceptibility and prognosis, and to predict response to treatment	Screening for the *HLA-B*1502* polymorphism in patients with Han Chinese, Thai, Indian and Malaysian ancestry prior to starting carbamazepine or phenytoin
Personalised medicine	Treatments targeted to the needs of individual patients based on genetic, biomarker, phenotypic or psychosocial characteristics	Avoiding levetiracetam in patients with psychiatric co-morbidities; using topiramate or zonisamide in obese epilepsy patients
Precision medicine	Treatments targeted at the underlying disease mechanism, most commonly molecular-genetic factors	Ketogenic diet for GLUT1 deficiency syndrome; everolimus for seizures in tuberous sclerosis complex

1.1 Defining seizures and epilepsy

Seizures are spontaneous episodes of synchronous increases in neuronal activity in the brain, leading to transient clinical signs and symptoms. The propensity for recurrent unprovoked seizures defines epilepsy. Previously, the diagnosis of epilepsy required two or more unprovoked seizures occurring at least 24 hours apart[11]. In 2014, the International League Against Epilepsy (ILAE) amended the operational definition of epilepsy, such that epilepsy can now be diagnosed after a single seizure if the estimated risk of seizure recurrence within the next 10 years is at least 60%[12].

Epilepsy is not a singular disease entity but rather a spectrum of disorders, "*the epilepsies,*" with varied seizure types, heterogeneous aetiologies and many co-morbidities. Conventionally, the epilepsies are dichotomised into distinct focal and generalised categories. Focal epilepsies are characterised by seizures that emanate from one or multiple (multifocal) brain regions. Generalised epilepsies comprise seizures with initial neuronal activation involving both cerebral hemispheres. Recognising that this dichotomy was overly simplistic, the ILAE proposed a multi-layered framework for the classification of the epilepsies. The revised framework incorporates seizure types, epilepsy types, epilepsy syndromes, aetiology, and co-morbidities (see **Figure 1.1**)[13, 14].

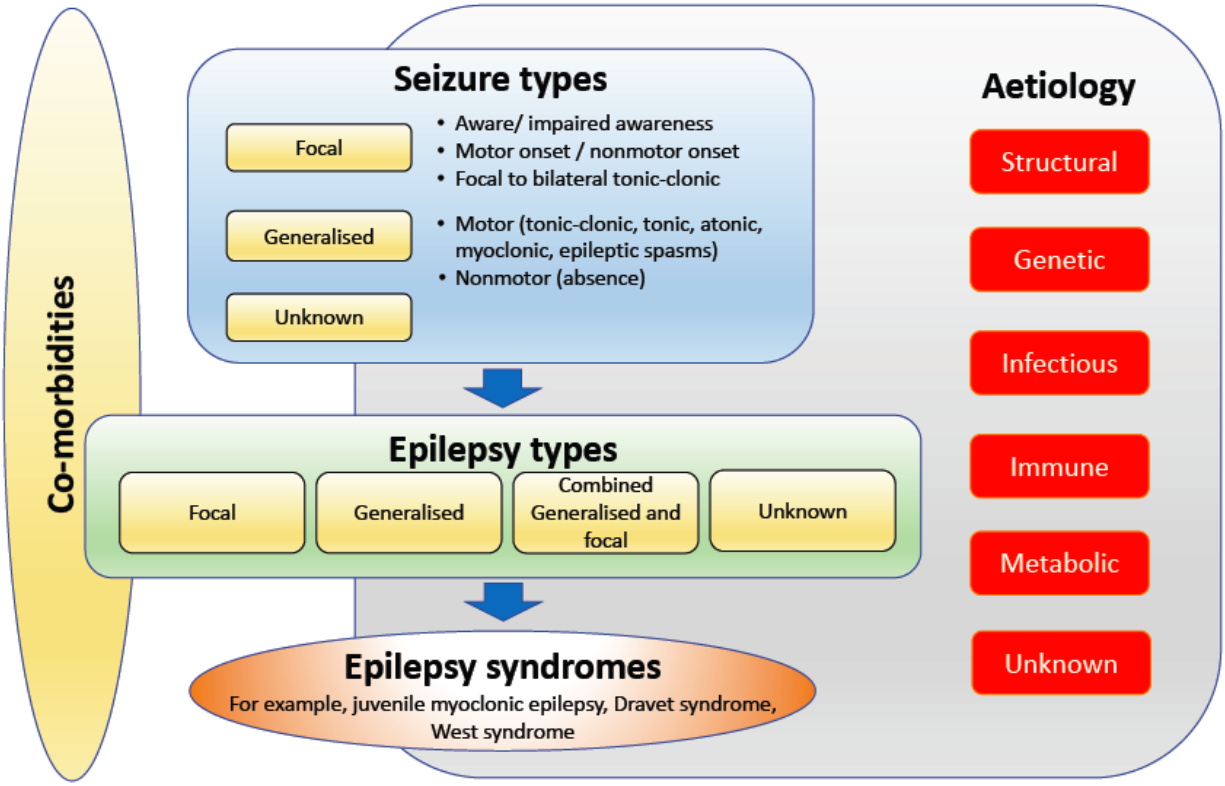

Figure 1.1 The ILAE multi-layered classification of the epilepsies.

Image adapted from Scheffer *et al*, 2017 and Fisher et al, 2017[13, 14].

1.2 Epilepsy epidemiology rooted in an Irish context

Epilepsy is one of the most common neurological disorders, affecting more than 50 million people worldwide. Using epidemiological markers such as disability-adjusted life-years (DALYs), years of life lost and years lived with disability, it is estimated that epilepsy accounts for approximately 0.5% of the global burden of disease[15]. Epilepsy was the fifth leading cause of neurological-related DALYs lost, behind stroke, migraine, dementia and meningitis[15]. The estimated monetary cost of epilepsy to the European economy was over €15 billion in 2004, based on calculations that included costs relating to health and social care provision, and indirect costs such as sick leave. The estimated societal cost of epilepsy in Ireland amounted to €146 million in 2004[16]. Epilepsy epidemiological studies are hampered by differences in epilepsy definitions and classifications, problems with case ascertainment and the heterogeneity of epilepsy aetiologies. In most epidemiological studies, epilepsy is defined by the presence of more than one unprovoked seizure. A meta-analysis of international epidemiological studies estimated the point prevalence of active epilepsy to be 6.38 per 1000 persons[17].

Recent studies have thoroughly investigated the epidemiology of epilepsy in Ireland. A study by Linehan *et al* in 2010, estimated the prevalence of epilepsy in Ireland using data collected from multiple sources[18]. Analysis of ASM prescriptions from the Primary Care Reimbursement Services (PCRS) database found that between 33,000 and 36,000 persons aged five years or older were treated for active epilepsy. However, it is important to note that PCRS data may not capture patients who choose to remain untreated for epilepsy, or those who are prescribed ASMs for other reasons, such as headache or psychiatric disorders (for example, lamotrigine for bipolar affective disorder). According to survey data, 10 per 1000 Irish adults self-reported that they had received an epilepsy diagnosis from a doctor[18]. However, self-report data may underestimate true prevalence rates of epilepsy due to issues related to disclosure, as epilepsy is known to be associated with stigma and discrimination.

A recent prospective study estimated the incidence of first seizures and new epilepsy diagnosis in Cork, a county in southern Ireland with a population of 542,869 people[19].

In this comprehensive analysis employing multiple methods of case ascertainment, the incidence of first seizures was 102 per 100,000. Using the latest ILAE definition of epilepsy (multiple unprovoked seizures or a single unprovoked seizure and a greater than 60% risk of recurrence within 10 years) the incidence of new diagnosis of epilepsy was 62 per 100,000, compared with 41 per 100,000 when the 1993 ILAE epilepsy definition was applied[11]. Seventy-one percent of new epilepsy diagnoses were classified as focal, 11% as generalised and the epilepsy type was unknown in 18% of patients. The annual incidence of first seizures and new diagnosis of epilepsy had a bimodal distribution, with peaks in the first year of life and after 70 years. Cerebrovascular disease and brain tumours were the most common causes of epilepsy in the later peak[19].

The incidence of first seizures and newly diagnosed epilepsy was highest in the most socially deprived areas of Cork[20]. Few cases had a confirmed genetic diagnosis, limiting the investigation of genetics as a potential contributing factor. Maloney *et al* concluded that the higher incidence of epilepsy in poorer areas was likely multifactorial, and that factors associated with living in socially deprived areas put people at increased risk of developing epilepsy[20].

1.3 Mortality in epilepsy

People with epilepsy (PWE) have a higher mortality rate compared to the general population. In a Finnish cohort study of children diagnosed with epilepsy in 1964, almost one-quarter (60/245) of participants died during a 40-year observation period. The mortality rate was three times higher than a matched control population, with sudden unexpected death in epilepsy (SUDEP) the most common cause of death[21]. Mortality in epilepsy can be divided into three categories: (a) deaths directly related to epilepsy; (b) deaths indirectly related to epilepsy; and (c) deaths due to the underlying neurological disease (see **Table 1.2**)[22].

Table 1.2 Causes of epilepsy-related mortality

Deaths directly due to epilepsy	Deaths indirectly due to epilepsy	Deaths due to the underlying neurological disease
• SUDEP • Status epilepticus • Drowning • Motor vehicle accident • Falls, burns and crush injuries	• Aspiration pneumonia • Suicide • Cardiovascular disease exacerbated or caused by ASM treatment • Serious ASM adverse events	• Brain tumour • Stroke • Genetic or metabolic disease • Neurodegenerative disease

Table adapted from Devinsky *et al,* 2016[22].

1.3.1 Sudden unexpected death in epilepsy

SUDEP and status epilepticus (SE) are the most common causes of death directly related to epilepsy. SUDEP refers to the "*unpredictable and unanticipated death of a reasonably healthy person with epilepsy, where no cause of death can be found*" after post mortem examination[23]. The incidence of SUDEP ranges from 0.09 per 1000 person-years in prospective studies of newly diagnosed epilepsy, to 9.3 per 1000 person-years in patients with drug-resistant epilepsy (DRE) referred for epilepsy surgery consideration[24-26]. SUDEP risk factors include uncontrolled bilateral tonic-clonic seizures (generalised and focal-onset), earlier age of epilepsy onset, longer epilepsy duration, DRE, ID, nocturnal seizures, living alone, and seizures in the prone position[23, 26, 27]. Nocturnal supervision is associated with reduced risk of SUDEP[28]. The

mechanisms underlying SUDEP are complex and likely multifactorial. Evidence from witnessed and video-electroencephalography (vEEG) monitored SUDEP cases suggest that seizure-induced hypoventilation and apnoea play an important role in SUDEP pathophysiology[29, 30], with autonomic cardiac dysfunction also implicated[31].

Genetic variation likely contributes to SUDEP risk, probably involving complex polygenic factors. Analysis of exome sequencing data found an increased polygenic burden of deleterious variants in 18 people who died of SUDEP compared with 87 living epilepsy controls[32]. Some genetic epilepsies are associated with an increased risk of SUDEP. Dravet syndrome is a severe epilepsy syndrome characterised by prolonged early life febrile and afebrile seizures of varying semiology, with later emergence of developmental delay and refractory multifocal epilepsy. It is caused by pathogenic variants in the *SCN1A* gene in 80% of cases, leading to loss-of-function (LoF) of neuronal $Na_v1.1$ sodium channels[33]. In a longitudinal study of 100 patients with Dravet syndrome, 17 died after median follow-up of 10 years, with SUDEP accounting for 59% of deaths[34]. The increased risk of SUDEP in individuals with Dravet syndrome may be due, in part, to the frequent, severe seizures that are characteristic of the disorder. Additionally, autonomic and cardiac dysfunction may also contribute to SUDEP risk in Dravet syndrome. Patients with Dravet syndrome have decreased heart rate variability, a marker of altered autonomic tone associated with sudden cardiac death[35]. *Scn1a* knockout mice exhibit ictal bradycardia before fatal seizures, which may be linked to involvement of vagal parasympathetic pathways[36].

An exome-based analysis of confirmed SUDEP cases found clinically relevant mutations in cardiac arrhythmia and epilepsy genes in 46% (28/61) of cases, including four pathogenic variants in long QT syndrome genes (*KCNH2*, *KCNQ1* and *SCN5A*). Pathogenic variants in the epilepsy gene *DEPDC5* were identified in six SUDEP cases[37]. SUDEP risk in epilepsy patients with *DEPDC5* variants will be discussed in later chapters.

1.3.2 Status epilepticus

SE is a medical emergency with significant morbidity and mortality. As most seizures spontaneously abort within 1-2 minutes, the ILAE define convulsive SE as continuous seizure activity for more than 5 minutes. Two or more convulsive seizures with incomplete recovery in between is also considered SE[38]. In a meta-analysis of published SE mortality studies, the pooled mortality rate was 14.9%, with higher case fatality amongst elderly patients (24.9%)[39]. The most important determinant of SE-related mortality is the underlying cause. Acute symptomatic causes (stroke, encephalitis, trauma) of SE are associated with higher mortality compared with chronic epilepsy[40]. Over 60% of SE cases can be attributed to acute symptomatic aetiologies[41].

Around one in ten PWE present with SE as their first clinical manifestation[41]. Re-emergence of SE in PWE is often triggered by ASM nonadherence and/or subtherapeutic ASM serum concentrations[42]. Specific genetic aetiologies are strongly associated with SE, including Dravet syndrome and *POLG*-related epilepsies. Genetic aetiologies should be considered in refractory cases, as more targeted treatment strategies may be needed to control seizure activity (sodium channel-blocking ASMs like phenytoin may exacerbate seizures in Dravet syndrome) and to avoid devastating complications (valproate-associated hepatotoxicity in *POLG*-related disorders)[43].

1.4 The hidden disability of epilepsy

Historically, the morbidity of epilepsy was largely attributed to seizures and their consequences. Generalised-onset seizure types such as tonic-clonic, tonic and atonic seizures, and focal seizures with impaired awareness often cause bone fractures, joint dislocations, crush injuries, lacerations, tooth injuries, burns, and head trauma. Seizures and related injuries account for approximately 1% of all emergency department attendances, and 3% of all prehospital ambulance transports[44]. Risk factors for seizure-related injuries include higher seizure frequency, greater ASM number, less independent living situation, and history of tonic-clonic seizures or drop attacks[45].

More recently, greater emphasis has been placed on the 'hidden disability' of epilepsy. PWE struggle to find employment. In a prospective cohort study from Denmark, patients with childhood-onset epilepsy had lower educational level, higher unemployment rates and lower incomes compared with matched controls[46]. Predictors of employment include normal intelligence, onset of epilepsy after the age of six years, and uninterrupted seizure remission[47]. Employers cited fears about work-related accidents and concerns around customers witnessing seizures as deterrents to hiring PWE, highlighting the impact of workplace stigma on high unemployment rates for PWE[48].

Cognitive dysfunction is probably the most important determinant of employment for PWE. In a Swedish study of 713 adults with active epilepsy, 23% had co-morbid ID[49]. ID and/or autism spectrum disorder (ASD) may be part of complex neurodevelopmental disorders, of which epilepsy is an additional clinical manifestation. Developmental and epileptic encephalopathies (DEE) are a group of severe epilepsies, in which cognitive impairment is influenced by both uncontrolled seizure activity and the underlying cause[50]. *De novo* genomic variants not inherited from either parent are an important cause of DEE[51].

Cognitive deficits caused by acquired structural aetiologies such as stroke and traumatic brain injury are usually static and irreversible. Recurrent or prolonged

seizures, ASM complications, and epilepsies involving hippocampal structures may lead to dynamic cognitive disturbances, which may be reversible[52]. Psychiatric disorders affect one in three PWE, with mood disorder and psychosis particularly prevalent in epilepsies involving the mesial temporal lobes[53].

Psychogenic non-epileptic seizures (PNES, also known as dissociative seizures or functional seizures) are attacks of altered subjective experience and/or involuntary movements that resemble epileptic seizures, but are not caused by abnormal electrical activity in the brain. Instead, PNES result from complex neuropsychiatric dysfunction. PNES is a common disorder, accounting for 11% of 'convulsive seizure' presentations to emergency departments[54], and approximately one-third of vEEG monitoring admissions[55]. The gold standard investigation for differentiating PNES from epilepsy is vEEG. PNES are associated with significant morbidity and mortality. Fifteen percent of patients with PNES experience prolonged seizure episodes (PNES-status) leading to intensive care unit admission, of whom 41% receive oral intubation[56]. A retrospective cohort study from Australia found that PNES without co-morbid epilepsy had a mortality rate 2.5 times greater than the general population, similar to patients with DRE[57]. Suicide and accidental poisonings accounted for 16.4% of deaths. Patients with PNES had elevated rates of death from heart disease, diabetes and chronic obstructive airway disease, indicating that causes of excess mortality were largely preventable[57]. Treatment of PNES involves careful explanation of the diagnosis, stopping unnecessary ASMs, psychoeducation and psychotherapy. Co-morbid epilepsy and PNES is not uncommon, and presents significant diagnostic and therapeutic challenges. In a study from the Beaumont Hospital epilepsy team, one-third of patients with PNES diagnosed by vEEG had coexisting epilepsy[55].

Our therapeutic efforts in epilepsy are heavily focused on controlling seizures. Seizure freedom is the ultimate therapeutic goal for PWE, as it can have a significant impact on quality of life, including the ability to drive and find employment. Seizure freedom consistently correlates with improved quality of life[58, 59]. However, seizure freedom may be unattainable for some patients with severe epilepsies, like DEE. Striking a balance between therapeutic efficacy and reduced side-effects, with palliative goals like controlling dangerous seizures (bilateral tonic-clonic seizures or SE) or reducing the overall ASM burden may lead to improved quality of life for people with severe

DRE. A key goal of precision medicine in the genetic epilepsies is to develop disease-modifying treatments that not only target seizures, but also the intrinsic cognitive and psychiatric disturbances associated with these disorders.

1.5 Drug-resistant epilepsy

After outlining the considerable morbidity and mortality of epilepsy, it is worth highlighting that over two-thirds of PWE achieve good seizure control on ASM therapy. Remission is achieved after the first or second ASM trial in ~90% of seizure-free patients. However, the likelihood of seizure freedom diminishes exponentially with sequential unsuccessful ASM trials[60]. Accordingly, the ILAE define DRE as failure to control seizures after "*adequate trials of two tolerated and appropriately chosen and used ASM schedules, whether as monotherapies or in combination*"[61]. The rate of DRE has stubbornly remained at 30% despite the introduction of more than a dozen new ASMs in the past two decades[62].

The negative consequences of DRE are manifold. Uncontrolled epilepsy has a detrimental effect on quality of life, influenced by driving restrictions and limited employment opportunities[63]. DRE is an important risk factor for SUDEP[23, 27]. Cognitive deficits are common in DRE, related to frequent seizures, interictal epileptiform activity and ASM complications[52]. The economic impact of DRE at a societal level is considerable, due to high healthcare use and indirect costs such as reduced productivity at work. Annual healthcare-related costs for patients with DRE in the USA were 2.3 times higher than patients with well-controlled epilepsy ($12,399 versus $5,511)[64]. A modelling analysis on the cost of epilepsy in Australia predicted that DRE results in lost gross domestic product of US $22.1 billion. The model predicted retention of US $2.6 billion if seizure freedom rates increased by 5%[65]. Frequently reported predictors of DRE include younger age of onset, abnormal electroencephalogram (EEG), focal seizures, failure to respond to first ASM, developmental delay and psychiatric co-morbidities[66]. Polymorphisms in the *ABCB1* gene are also associated with ASM resistance[67].

1.5.1 Treatment approaches in drug-resistant epilepsy

In focal-onset DRE, epilepsy surgery offers the greatest likelihood of seizure freedom[68]. The best candidates for resective surgery have a single epilepsy focus based on concordant clinical, imaging and vEEG data. Over 60% of patients with mesial temporal lobe epilepsy achieve seizure freedom from temporal lobectomy[69].

The introduction of high resolution (3 Tesla and higher) magnetic resonance imaging (MRI), intracranial EEG, fluorodeoxyglucose (FDG)- positron emission tomography (PET) imaging, and ictal single-photon emission computed tomography (SPECT) has improved surgical outcomes in non-lesional and extra-temporal epilepsies, extending the option of epilepsy surgery to more patients[70, 71]. Despite these advances, a substantial proportion of DRE patients remain ineligible for epilepsy surgery and one-third have persistent seizures after surgery[72].

Neurostimulation procedures like vagus nerve stimulation (VNS), deep brain stimulation (DBS) and responsive neurostimulation (RNS) are options for DRE when resective surgery is contraindicated, although overall seizure freedom rates are less impressive compared with surgery[73]. The recently approved ASM cenobamate appears to be more effective than other ASMs for focal-onset DRE, with a seizure freedom rate of 13% in one long-term extension study[74]. Despite high response rates compared to other ASM trials, most participants treated with cenobamate still experienced seizures. Therefore, the search for more efficacious treatments for DRE remains a priority.

1.5.2 Tackling drug-resistant epilepsy with better aetiologic classification

Identifying the underlying cause of DRE and establishing rational aetiologic-based treatments may be a more effective therapeutic strategy for intractable epilepsies. This is reflected in the latest ILAE classification of the epilepsies, which emphasised the importance of early identification of structural, genetic, immune, infectious, and metabolic aetiologies[14]. The revised classification framework prioritises identification of aetiologies that can be targeted with specific treatments.

Advances in diagnostic technologies have enhanced our ability to accurately classify epilepsies based on aetiology. The wider availability of gene panels, whole exome sequencing (WES) and whole genome sequencing (WGS) have led to increased detection of monogenic epilepsies. Diagnostic developments are not limited to genetics. Previously MRI-invisible malformations of cortical development (MCD) such as focal cortical dysplasia (FCD), are now detectable using high-field MRI. Progress in detection of neural antibodies against cell surface and intracellular antigens has heightened awareness of autoimmune causes of epilepsy. A small proportion of DRE

is immune-mediated, with better response to immunotherapies compared with ASMs[75]. Some epilepsies fall within multiple aetiologic classifications (see **Figure 1.2)**. For example, epilepsy in tuberous sclerosis complex (TSC) has both genetic and structural aetiology. The structural aetiology is relevant for epilepsy surgery consideration, while the genetic aetiology has implications for precision therapy with mechanistic (formerly known as mammalian) target of rapamycin (mTOR) inhibitors, like everolimus[14].

Drug-resistant epilepsy in tuberous sclerosis complex

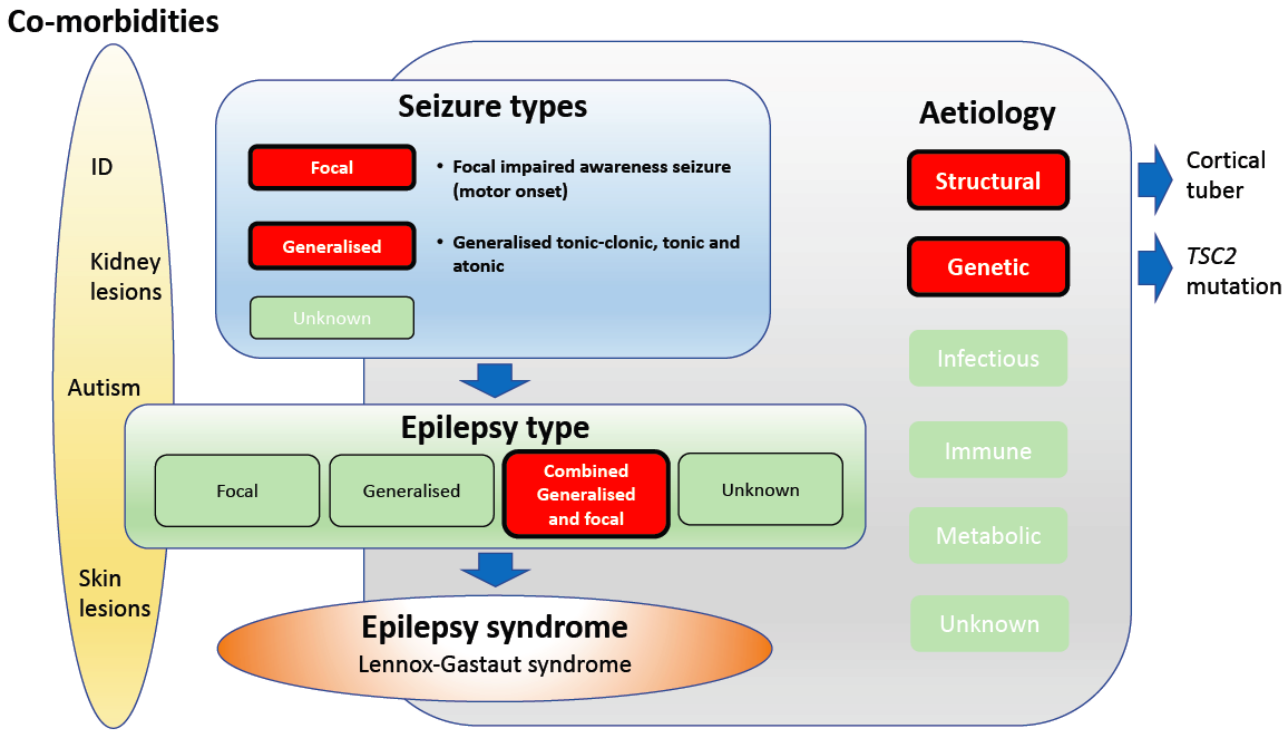

Figure 1.2 Aetiologic classification of tuberous sclerosis complex using the ILAE 2017 framework[14].

Despite advances in imaging and genetics, aetiologic classification remains elusive in many patients with epilepsy. In a retrospective analysis of 3,216 epilepsy patients attending the epilepsy clinic at Beaumont Hospital, the aetiology was unknown in 46% of patients (see **Figure 1.3**), highlighting the importance of ongoing research to better understand the full spectrum of epilepsy aetiology[76]. Improved aetiologic classification is necessary to implement a precision medicine approach, particularly for genetic epilepsies. Precision diagnostics involving advanced bioinformatic analysis of NGS and high-resolution imaging data will inevitably improve diagnostic yield.

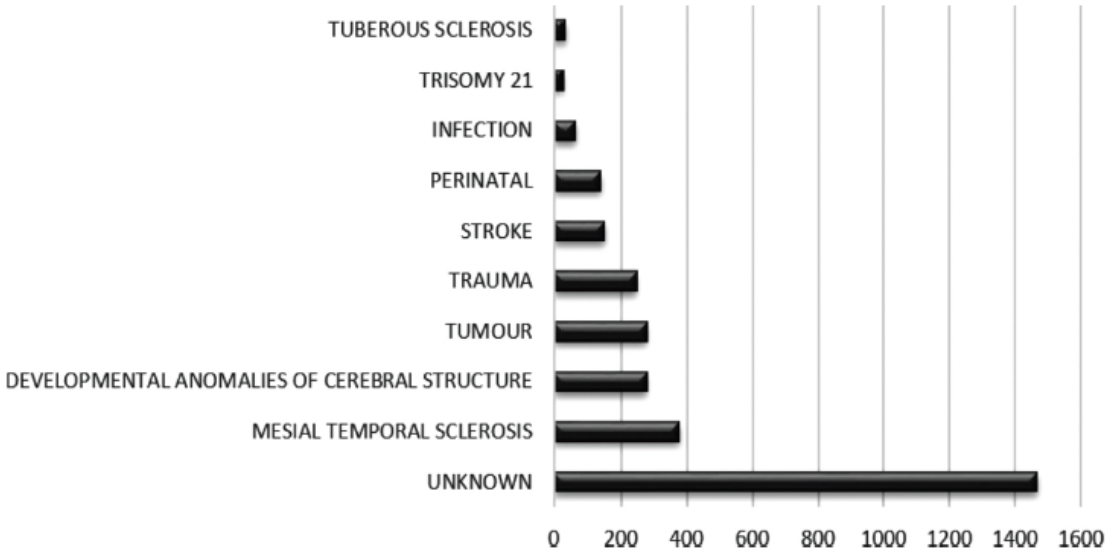

Figure 1.3 Overall breakdown of the most common epilepsy aetiologies in patients attending Beaumont Hospital.

Figure used with permission from Delaney *et al*, 2020[76].

1.6 Epilepsies with genetic aetiologies

1.6.1 Introduction

The remainder of the thesis will focus on epilepsies with genetic causes. A growing number of genomic variants have been causally associated with a wide spectrum of disorders in which epilepsy is a major component. In a prospective cohort study conducted in Scotland, the annual childhood incidence of monogenic epilepsies was estimated to be around 1 per 2,000 live births, with pathogenic variants in eight genes accounting for 80% of cases[77]. Monogenic disorders are caused by damaging germline variants (i.e., deoxyribonucleic acid [DNA] changes present in sperm or ovum cells) in single genes, and follow Mendelian inheritance patterns (autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive). *De novo* mutagenesis is an important mechanism in severe epilepsies, like DEE. Monogenic epilepsies are frequently drug-resistant, and often have debilitating neurodevelopmental complications. Individually rare but collectively common, these disorders place considerable strain on clinical resources.

Common epilepsies such as the genetic generalised epilepsies display complex non-Mendelian inheritance, with contribution from multiple common variants of small effect size, similar to type 2 diabetes and schizophrenia[78]. There is growing interest in the contribution of somatic mosaicism to the pathogenesis of lesional epilepsies, such as FCD type II. Somatic mutations are DNA changes acquired after conception (post-zygotic) (see **Figure 1.4**). Somatic mutations can occur in any cell type except germ cells. Somatic variants affecting a subset of neural cells (mosaicism) can give rise epileptogenic cortical malformations[79]. We now know that genetic factors underlie a substantial proportion of epilepsies, with contribution from rare damaging variants in severe epilepsies, polygenic common variation in genetic generalised epilepsies, and somatic mosaicism in lesional epilepsies.

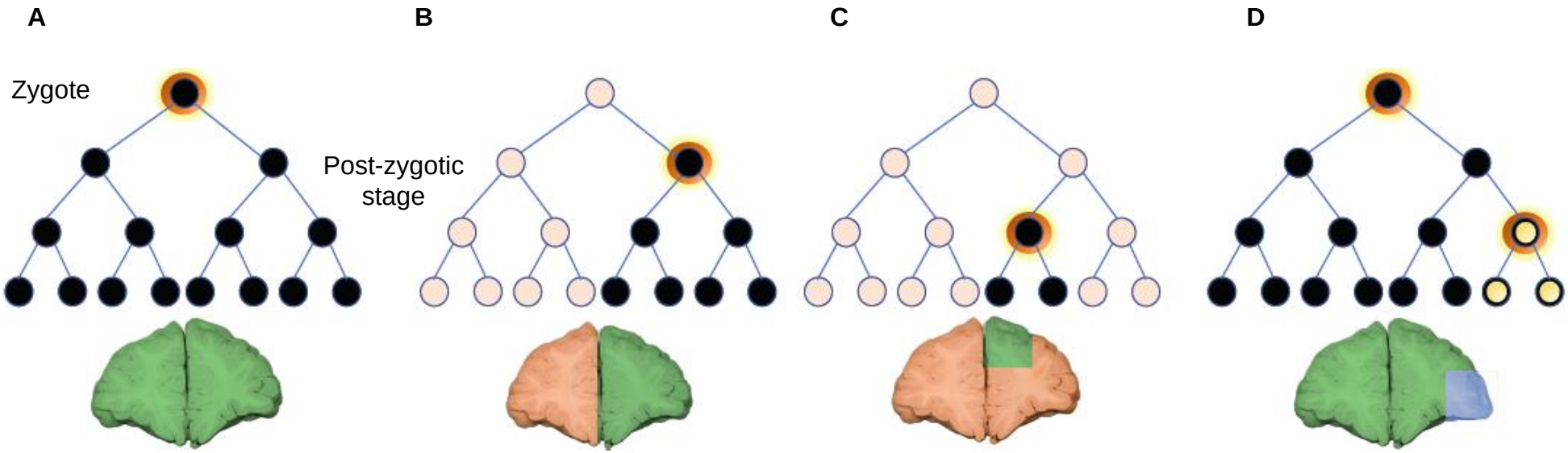

Figure 1.4 Germline and somatic mutagenesis in the epilepsies

Image **A** indicates a germline mutation, a heritable change in DNA occurring in a germ cell (sperm or ovum). Germline mutations are present in every cell in the body. Image **B** depicts an early post-zygotic somatic mutation in the *MTOR* gene causing extensive hemispheric cortical malformation. Image **C** illustrates a late post-zygotic somatic mutation in *MTOR* causing focal cortical dysplasia. Image **D** denotes a patient with a germline mutation in *DEPDC5* affecting every cell in the brain. A second-hit somatic variant in *DEPDC5* leads to focal cortical dysplasia.

1.6.2 Genetic testing in the epilepsies

Advances in NGS approaches and associated data analytics have accelerated gene discovery in the epilepsies. NGS enables large-scale unbiased genomic investigation of all protein-coding sequences (exome, 1-2% of the genome) or the entire genome (all coding and non-coding sequences). Sequencing approaches, such as targeted gene panels, WES and WGS are increasingly used in neurology clinical practice[80]. Targeted gene panels examine a select set of genes with known associations to a phenotype or disease (see **Table 1.3**). WES and WGS enable detection of variants in known 'disease genes,' including genes not known to be associated with the phenotype. Sequencing approaches without predefined target genes present biostatistical challenges related to abundant candidate genetic variants of uncertain significance (VUS), and ethical quandaries when secondary findings are uncovered[80]. In the pre-NGS era, the choice of genetic test was guided by the epilepsy phenotype. Sanger sequencing or multiplex ligation probe amplification (MLPA) were used to investigate genes or genomic regions known to be associated with a particular phenotype. This narrow approach is effective for genetic disorders with well characterised phenotypes, like TSC. More comprehensive, unbiased genomic investigation is preferable for heterogeneous disorders such as epilepsy and autism. *De novo* mutation discovery in DEE has prospered due to the trio-study paradigm (proband and parents sequenced). Candidate *de novo* variants are readily identified by excluding all variants inherited from either parent[51].

Copy number variants (CNV) are deletions or duplications of genetic information larger than one kilobase. There are two major classes of CNV: recurrent and private[81]. Recurrent CNVs occur at genomic hotspots prone to rearrangement. Three recurrent deletions (15q13.3, 15q11.2 and 16p13.11) are risk factors for epilepsy, with 3% of patients with genetic generalised epilepsies carrying at least one of these deletions[82]. These recurrent CNVs are also associated with an increased risk of ID, ASD and schizophrenia[83]. In contrast, private CNVs are rare and can occur at any genomic region. Large private CNVs, affecting many genes are an important cause of DEE[84]. Wolf-Hirschhorn syndrome is a distinctive neurodevelopmental disorder caused by 4p16.3 deletion, in which epilepsy is a prominent feature[85]. CNVs involving established epilepsy genes like *SCN1A* cause DEE phenotypes[86]. Comparative genomic hybridization (CGH) microarray analysis is currently the best available test to identify

CNVs (see **Table 1.3**), although WGS-based CNV detection has the potential to supersede microarray analysis[87]. Karyotyping for diagnosis of chromosomal abnormalities has largely been replaced by microarray analysis, although karyotyping is still required to detect ring chromosome 20. Routine WGS-based techniques can also miss imprinting disorders, such as Angelman syndrome[88]. Angelman syndrome is caused by a large deletion of 15q11q13 on the maternal chromosome in 75% of cases. However, 3% of cases are caused by an imprinting defect without an associated deletion, while another 1-2% of cases are caused by paternal uniparental disomy (two copies of chromosome 15 inherited from the father). Methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA) is the recommended first line molecular test in cases of suspected Angelman syndrome[88]. Rare repeat expansion disorders known to cause epilepsy (for example, progressive myoclonic epilepsy of Unverricht-Lundborg type and familial adult myoclonic epilepsy) can also be missed by clinically available NGS approaches[89].

In the epilepsies, sequencing efforts are most commonly directed towards the detection of germline variants using lymphocyte-derived DNA, obtained from peripheral blood samples. NGS techniques are increasingly used to detect somatic variants in brain tissue obtained during epilepsy surgery. Variant allele frequency (VAF) is a metric used to estimate the proportion of cells in a sample carrying a specific variant. It is common for patients with FCD type II to have low VAF values, with 80% having values less than 5%[90]. Accurate identification of low-level somatic mosaicism requires high-depth sequencing and advanced bioinformatics analysis, followed by independent validation by droplet digital polymerase chain reaction (PCR), targeted amplicon sequencing or Sanger sequencing[90, 91]. Single cell sequencing of micro-dissected dysmorphic neurons and balloon cells may improve detection of somatic mutations in FCD, as these cell types are the main carriers of mutations in FCD[90]. A recent ILAE consensus statement on the classification of FCD recommended deep sequencing analysis of brain tissue to establish genetic aetiology[92].

Table 1.3 Genetic testing approaches in epilepsy

Test	Detects	Indication
Single gene sequencing	Variants in a single gene	Strongly suspect a specific genetic diagnosis (for example, *SCN1A* variant in Dravet syndrome)
Gene panel	Variants in a predefined set of genes associated with a given phenotype	Epileptic encephalopathy, epilepsy with ID or autism, refractory non-lesional epilepsy, epilepsy associated with specific brain lesions
Exome sequencing	Variants in all protein-coding DNA	Same as gene panel
Genome sequencing	Variants in all protein-coding and non-coding DNA	Same as gene panel *(Rarely used in clinical settings outside the United Kingdom)*
Comparative genomic hybridization microarray	Copy number variants	Epilepsies with co-morbid ID, autism or dysmorphic features
Karyotyping	Large chromosomal abnormalities	Suspected ring chromosome 20
Methylation-sensitive multiplex ligation-dependent probe amplification	Methylation status and large chromosomal deletions	Suspected Angelman syndrome
Repeat-primed PCR	Repeat expansion disorders	Suspected progressive myoclonic epilepsy (*EPM1* dodecamer expansion)
Human leukocyte antigen typing	Risk alleles associated with hypersensitivity to carbamazepine	Patients with Han Chinese or South Asian ethnicity prior to treatment with carbamazepine
Targeted deep exome sequencing	Low-level somatic mosaic variants in predefined genes	Brain tissue obtained during epilepsy surgery

Abbreviations:
DNA= deoxyribonucleic acid; ID= intellectual disability; PCR= polymerase chain reaction

1.6.3 Genomic variant interpretation in the clinic

The American College of Medical Genetics and Genomics (ACMG) developed classification guidelines for the interpretation of sequence variants in genes known to cause Mendelian disorders[93]. ACMG guidelines recommend a five-tiered system for determining variant pathogenicity incorporating multiple lines of evidence, including population data, disease databases, *in silico* predictors, functional analysis, segregation data, *de novo* status, allelic data for recessive disorders (i.e., variants *in trans*), scientific and medical literature, and gene-specific phenotypes. Available evidence is weighted using standardised methodology, and variants are classified as either: pathogenic; likely pathogenic; VUS; likely benign; or benign (see **Figure 1.5**). Pathogenic and likely pathogenic variants are considered disease causing and 'clinically actionable'. VUSs have insufficient evidence either for or against pathogenicity. Hence, their clinical impact is uncertain[93]. VUSs are a frequent outcome of genomic testing, posing new challenges to clinicians, including how to report VUSs to patients. Inaccurate variant classification may have wider consequences beyond the proband, including unnecessary cascade testing and worry for probands and their families.

A multidisciplinary team (MDT) approach is recommended to tackle the VUS dilemma. Specialist MDT input has been shown to improve diagnostic rates in rare diseases, including mitochondrial disorders[94]. Systematic discussion involving clinical geneticists, relevant clinical specialists, bioinformaticians and translational scientists facilitates accurate variant interpretation. The MDT may advise further testing on VUSs that warrant further investigation. The Association of Clinical Genomic Science (ACGS) developed a temperature scale to highlight VUSs with high level supporting evidence for pathogenicity (see **Figure 1.5**)[95]. Functional characterisation or segregation testing of 'hot' or 'warm' VUSs may facilitate re-classification. Sharing interpreted genomic variants in exchange databases like *ClinVar* can help solve VUSs. After submitting to *ClinVar*, the clinician or laboratory receives a report outlining differences in interpretation between the submitted variant and those already deposited in *ClinVar*[96]. Periodic re-interpretation and re-analysis of raw sequencing data is recommended for patients with negative WES and WGS studies.

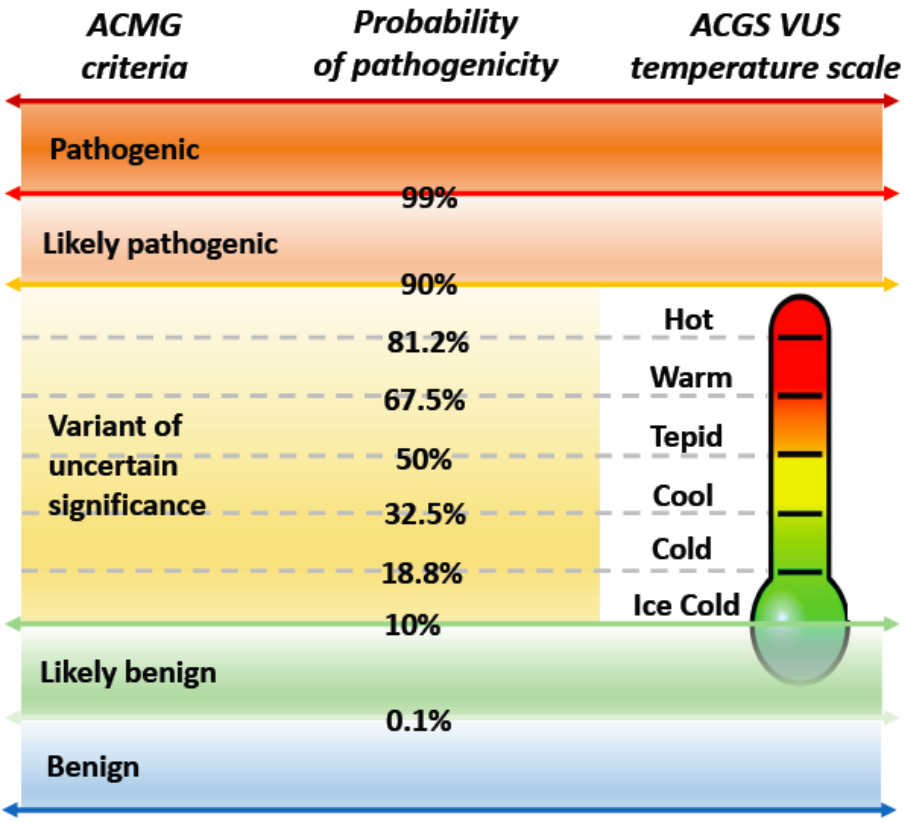

Figure 1.5 Variant interpretation criteria based on American College of Medical Genomics guidelines and the variant of uncertain significance temperature scale proposed by the Association for Clinical Genomic Science.

Figure adapted from Ellard *et al,* 2020[95].

1.6.4 Genetic developmental and epileptic encephalopathies

Prior to the advent of NGS, the aetiology of 'epilepsy with ID' was generally unknown and often misattributed to pre- or perinatal insults. Managing these severe epilepsies was accompanied by a sense of therapeutic nihilism. Now, a causative pathogenic variant is detected in upwards of 40% of epilepsy patients with co-morbid ID[9, 10, 97], opening novel avenues for therapeutic intervention.

Cognitive and behavioural impairments in DEE result from the combination of uncontrolled seizure and epileptiform activity, and the underlying disease biology[50]. DEE can have acquired aetiologies (for example, perinatal stroke and early life meningitis), as well as non-acquired structural causes, like MCD. Extensive MCD causing DEE is often genetic (for example, lissencephaly "smooth brain" caused by *LIS1* pathogenic variants)[98]. Some genetic DEEs have clinical features restricted to neurological domains (seizures, ID, ASD, motor deficits and movement disorders). DEEs with "brain-only" phenotypes include those caused by pathogenic variants in *STXBP1* or *KCNT1*[99]. Other genetic DEEs have multisystem manifestations, such as TSC. Rather than presenting an exhaustive list of the many genetic causes and clinical features of DEE, I will provide an overview of Dravet syndrome to highlight important concepts related to the genetic architecture of DEE.

1.6.5 Dravet syndrome: the archetypal genetic developmental and epileptic encephalopathy

The clinical syndrome of severe myoclonic epilepsy in infancy was first described by Charlotte Dravet in 1978[100]. Children initially exhibit normal development. Seizures usually emerge within the first six months of life, often precipitated by fever. Initial seizures are often prolonged, classically presenting as hemiclonic SE with fever. Seizures continue and developmental delay becomes apparent in the second year of life. Other seizure types develop over time, including myoclonic, tonic-clonic and focal seizures. Most develop DRE and a characteristic crouch-gait[33].

Over 80% of Dravet syndrome patients have heterozygous pathogenic variants in *SCN1A,* mostly occurring *de novo*[101]. Dravet syndrome and other DEEs display genetic heterogeneity. Pathogenic variants in *PCDH19*, *GABRA1*, *GABRG2* and *KCNA2* account for a small proportion of cases within the Dravet syndrome

spectrum[99]. Phenotypic heterogeneity or 'pleiotropy', whereby pathogenic variants in the same gene produce different phenotypes, is common with genetic variants that cause DEE. Pathogenic variants in *SCN1A* cause a spectrum of disorders including self-limiting febrile seizures, familial hemiplegic migraine, generalised epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome. Gonadal mosaicism (mutation confined to the ovum or sperm) explains the rare occurrence of unaffected parents producing two or more children with Dravet syndrome[102].

Electrophysical studies of Dravet-associated *SCN1A* variants show LoF of voltage-gated sodium channels ($Na_v1.1$)[103]. LoF in neuronal $Na_v1.1$ sodium channels leads to reduced action potential firing in γ-aminobutyric acid (GABA)-ergic inhibitory interneurons. Diminished GABAergic firing shifts the balance toward excitation, leading to seizures and developmental delay[104]. This excitatory-inhibitory imbalance explains why sodium channel blocking ASMs such as carbamazepine, phenytoin and lamotrigine exacerbate seizures in Dravet syndrome. Recent functional work on *SCN1*A variants uncovered gain-of-function (GoF) effects in patients with specific phenotypes, including early infantile DEE with movement disorders and congenital arthrogryposis. In contrast to Dravet syndrome, these patients benefit from treatment with sodium channel blockers[105].

Historically, misperceptions regarding the value of genetic testing in adults with epilepsy and co-morbid ID led to underutilisation. Recent research from the FutureNeuro group showed that genetic testing had a yield of 27% in adult patients with epilepsy and co-morbid ID[10]. The utility of genomic testing in adult epilepsy patients with ID was also examined in a Danish cohort of 200 patients. Similar to the Irish study, a genetic diagnosis was established in 23% (46/200) of patients. A genetic diagnosis led to gene-specific treatment changes in 17% (11/46) of patients, of whom 91% (10/11) experienced reduced seizures or improved "general well-being"[106].

1.6.6 Genetic generalised epilepsies

The latest ILAE classification introduced the term "genetic generalised epilepsies" to describe epilepsies with generalised seizure types, generalised spike-wave on EEG, and presumed genetic aetiology[14]. The term "idiopathic generalised epilepsies" (IGE) is reserved for four overlapping electroclinical syndromes: childhood absence

epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and epilepsy with generalised tonic-clonic seizures alone[107]. IGE accounts for 15-20% of all epilepsies[108]. Patients with IGE experience generalised tonic-clonic, myoclonic and absence seizures. Age of onset typically ranges from 3-25 years. ID is not a feature of IGE. Brain imaging is normal and EEG shows 2.5 to 5.5 Hz generalised spike-wave discharges. Around 80% of IGE patients respond to broad spectrum ASM therapy (valproate, lamotrigine, levetiracetam or zonisamide) or ethosuximide for absence seizures. Other syndromes that fall within the generalised epilepsy spectrum include epilepsy with eyelid myoclonia, epilepsy with myoclonic absences, and epilepsy with myoclonic-atonic seizures[107].

Despite the revised ILAE terminology, the genetic architecture of genetic generalised epilepsies remains largely unsolved. Evidence from population-based aggregation studies and twin studies support a significant genetic contribution. The risk of developing epilepsy is six times higher in first-degree relatives of IGE patients compared to the general population[109]. Monozygotic twins are highly concordant for generalised spike-wave activity and generalised seizures[110]. The search for disease-causing Mendelian variants in IGE has mostly been unrewarding, with a few notable exceptions. Pathogenic variants in $GABA_A$ receptor genes (*GABRG2* and *GABRA1*) are very rarely identified in patients with IGE[89]. GLUT1 deficiency is a rare cause of early onset absence epilepsy. Pathogenic variants in *SCL2A1* disrupt glucose transport to the brain. The ketogenic diet improves seizure control in GLUT1 deficiency, by providing an alternative cerebral energy source through ketones[8].

Lacking high-impact single gene drivers of disease, genetic generalised epilepsies are polygenic disorders, with contribution from multiple alleles of small effect. Genome wide association studies (GWAS) aim to identify common single nucleotide polymorphisms that contribute to disease susceptibility. The ILAE Consortium of Complex Epilepsies identified 11 genomic loci associated with genetic generalised epilepsies after applying meta-analytic approaches to GWAS data. This expanded analysis involving 15,212 epilepsy cases and 29,677 controls, identified plausible candidate genes within loci involved in ion-channel function, transcription factors and vitamin B6 metabolism[111]. Polygenic risk scores (PRS) generate individual level risk estimates based on the cumulative effects of common variants derived from GWAS

data. Patients with genetic generalised epilepsies have a significantly higher burden of common epilepsy-associated variants, as quantified by PRS, compared to population controls and focal epilepsy patients[78]. Potential clinical applications of PRS include: (a) prediction on the risk of seizure recurrence after first seizure, and (b) prediction of epilepsy type after first seizure to guide treatment choices.

1.6.7 The genetics of focal epilepsy

Focal epilepsies have historically been regarded as non-genetic disorders, with greater emphasis placed on acquired aetiologies, like head injury and infection. However, multiple strands of evidence support significant genetic contributions to the focal epilepsies. Several distinctive familial focal epilepsy syndromes have been described, each with specific genetic aetiologies (see **Figure 1.6**).

Autosomal dominant (AD) sleep-related hypermotor epilepsy (formerly known as AD nocturnal frontal lobe epilepsy) is characterised by clusters of brief hyperkinetic and/or tonic seizures arising from sleep, often with bizarre semiology. Brain imaging is normal. Carbamazepine or oxcarbazepine are usually effective in controlling seizures. The first Mendelian epilepsy gene was discovered in a large Australian kindred with AD sleep-related hypermotor epilepsy in the mid-1990s. Linkage analysis implicated the nicotinic acetylcholine receptor alpha 4 subunit (*CHRNA4)* gene on chromosome 20q, and a missense variant in *CHRNA4* was found to segregate with affected family members[112]. Subsequently, missense variants in other nicotinic acetylcholine receptor genes (*CHRNA2* and *CHRNB2*) were found to cause AD sleep-related hypermotor epilepsy[113]. Two observational studies reported impressive seizure frequency reductions with transdermal nicotine treatment in patients with *CHRNA4* pathogenic variants[114, 115]. Mutated neuronal nicotinic receptors exhibit increased sensitivity to acetylcholine, causing altered GABAergic interneuron activity and seizure generation[116]. It has been hypothesised that exogenous nicotine desensitises mutated receptors, restoring normal physiology and reducing epileptic activity[115].

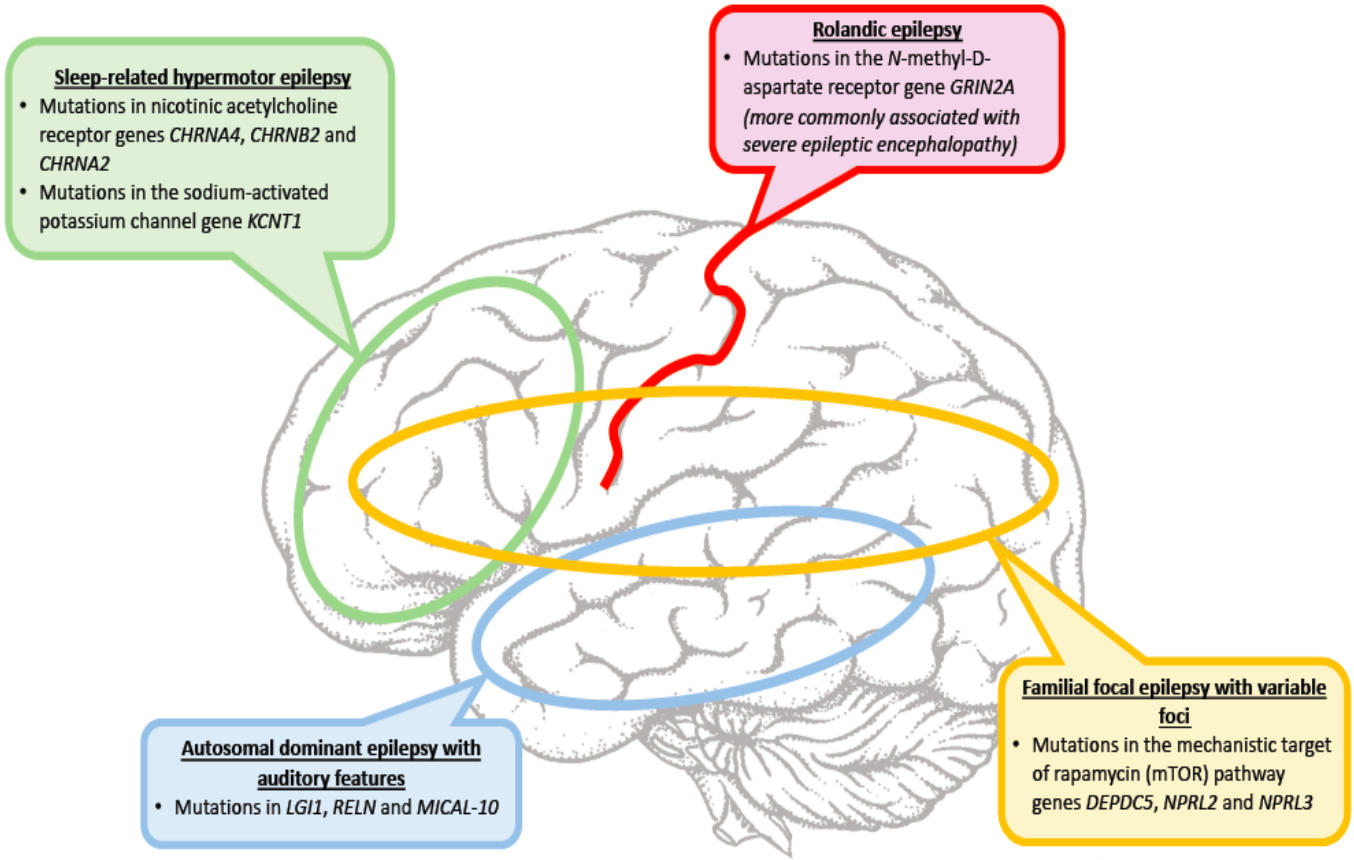

Figure 1.6 Monogenic causes of familial focal epilepsy syndromes.

The coloured circles represent the brain regions in which epileptic discharges predominantly arise in monogenic focal epilepsies. The corresponding coloured boxes contain the most common monogenic causes of these focal epilepsy syndromes. Figure adapted from Moloney *et al*, 2022[117].

Heterozygous pathogenic variants in the sodium-gated potassium channel gene *KCNT1* also cause sleep-related hypermotor epilepsy. Variants in *KCNT1* are highly pleiotropic, also causing severe phenotypes such as epilepsy of infancy with migrating focal seizures and West syndrome[118]. Quinidine showed early promise as a precision treatment for *KCNT1*-related epilepsy[119]. With mixed sodium and potassium channel blocking properties, quinidine seemed a rational treatment choice against *KCNT1* GoF channels. However, an observational study of 43 patients with *KCNT1*-related epilepsy found that only 20% benefited from quinidine and dose-limiting severe cardiac adverse events were common[120].

AD epilepsy with auditory features is a rare disorder characterised by focal seizures with auditory auras, reflex seizures triggered by specific sounds, and ictal aphasia. The semiology indicates seizure onset from the lateral temporal lobes. Brain MRI is normal and EEG occasionally shows epileptiform discharges arising from temporal regions. Epilepsy with auditory features has a relatively benign clinical course and most achieve seizure freedom with ASM treatment[121]. Pathogenic variants in *LGI1* are found in 30-50% of patients with familial epilepsy with auditory features[122, 123]. *LGI1*-related epilepsies most often occur in large pedigrees with many affected relatives, although sporadic cases have also been reported[124]. Penetrance refers to the proportion of people with a particular genetic variant that express an associated trait (the phenotype). Familial *LGI1* variants exhibit reduced penetrance, with variants inherited from unaffected parents in 67% of cases[125]. Pathogenic variants in *RELN* and *MICAL-1* also cause AD epilepsy with auditory seizures[123, 126].

Familial focal epilepsy with variable foci (FFEVF) is a dominant disorder, in which affected family members develop epilepsies emanating from different brain regions (i.e., one relative has frontal lobe epilepsy, another has temporal lobe epilepsy and another has seizures arising from occipital regions). Pathogenic variants in genes encoding subunits of the GTPase-activating protein (GAP) activity towards Rags 1 (GATOR1) complex cause FFEVF[127]. These epilepsies are collectively known as the GATOR1-related epilepsies. The GATOR1 complex comprises three subunits: Dishevelled, Egl-10 and Pleckstrin domain-containing protein 5 (DEPDC5); nitrogen permease regulator-like 2 (NPRL2); and nitrogen permease regulator-like 3 (NPRL3)[128]. *DEPDC5* variants were first identified as a cause of FFEVF in 2013[129].

Pathogenic variants in *NPRL2* and *NPRL3* were detected in FFEVF pedigrees in 2015[130]. The phenotypic spectrum includes sleep-related hypermotor epilepsy, epilepsy with auditory features, epilepsy caused by FCD type II or hemimegalencephaly (HME), infantile spasms, self-limiting epilepsy with centrotemporal spikes, and epilepsy with ID[127]. Importantly, over half with GATOR1-related epilepsies have DRE[127]. Several studies also report an increased risk of SUDEP in GATOR1-related epilepsies[37, 127].

GATOR1 complex inhibits mTOR pathway activity in response to cellular amino acid levels. Inactivating variants in *DEPDC5, NPRL2* and *NPRL3* cause epilepsy through mTOR pathway hyperactivity[131-134]. Everolimus is an approved treatment for epilepsy in TSC, the prototypical mTORopathy[135]. Given the shared neurobiology, we hypothesised that everolimus can ameliorate seizures in GATOR1-related epilepsies[136].

Other focal epilepsy syndromes with known genetic associations include self-limited neonatal epilepsy, self-limited neonatal-infantile epilepsy, self-limited infantile epilepsy and self-limited epilepsy with centrotemporal spikes. Self-limited neonatal epilepsy is characterised by focal-onset tonic seizures affecting head, face and limbs, first presenting between days two and seven of life. Seizures remit by six months, so long-term ASM treatment is not required. Heterozygous variants in two potassium channel genes (*KCNQ2, KCNQ3*) cause this phenotype[137]. Self-limited neonatal-infantile epilepsy is dominantly inherited, with seizure onset in the neonatal or infantile period. Focal-onset clonic or tonic seizures emerge between day two and seven months of life. Seizures are readily controlled by ASMs and remit by two years. This syndrome is caused by pathogenic variants in the sodium channel gene, *SCN2A*[137]. *KCNQ2* and *SCN2A* channelopathies demonstrate wide phenotypic pleiotropy, also causing severe DEE[99]. Self-limited infantile epilepsy is characterised by the onset of seizures in the infantile period (3-20 months), with a peak incidence at six months. Focal seizures are often frequent and may be difficult to control initially, but usually resolve by early childhood. The phenotypic spectrum includes paroxysmal kinesigenic dyskinesia/dystonia and other movement disorders. Brain imaging and development are normal[137]. Familial cases show autosomal dominant inheritance, with incomplete penetrance. Pathogenic variants in *PRRT2* are the most common genetic aetiology[77].

Self-limited epilepsy with centrotemporal spikes begins in early childhood, with brief focal seizures involving the tongue, throat and lower face. Seizures may progress to bilateral tonic-clonic seizures. EEG shows high-amplitude centrotemporal sharp-and-slow wave complexes, activated by drowsiness and sleep. Seizures tend to be responsive to ASMs and typically resolve by puberty. Affected children often have a family history of epilepsy, though monogenic causes are rarely identified, suggesting complex polygenic inheritance[138]. In rare cases, heterozygous pathogenic variants in *GRIN2A* cause self-limited epilepsy with centrotemporal spikes. However, *GRIN2A* variants are more commonly associated with severe phenotypes, including epileptic encephalopathy with spike-and-wave activation in sleep[139].

Large collaborative studies have shown that ultra-rare variants (absent in population databases like The Genome Aggregation Database, gnomAD) in genes known to cause rare familial epilepsy syndromes, also contribute to the genetic architecture of common epilepsies, like non-acquired focal epilepsies. Ultra-rare variation in *DEPDC5, LGI1*, *PCDH19*, *SCN1A* and *GRIN2A* contributed to the risk of epilepsy in 8% of patients with familial non-acquired focal epilepsy[140], while the Epi25 collaborative found weak enrichment of ultra-rare variants in a cohort of patients with mainly sporadic focal epilepsy[141].

1.6.8 Somatic mosaicism and lesional epilepsies

The previous section outlined the germline genetic contribution to non-lesional focal epilepsies. Brain lesions caused by abnormal cortical development are a major cause of focal epilepsies. Brain-confined somatic mutations play an important role in the pathogenesis of focal MCD. Using targeted deep sequencing techniques, somatic mTOR gene mutations were detected in almost 40% of FCD type II and HME brain specimens, of which two-thirds had mosaic *MTOR* variants[90, 91]. The timing of the somatic mutation correlates with the MCD size and variant load (i.e., earlier somatic mutations cause larger lesions with higher rates of mosaicism)[142]. Moreover, the level of mosaicism correlates with the degree of epileptogenicity[143]. Knudson's "two-hit" hypothesis explains how focal brain lesions develop in patients with germline mutations, as seen in TSC and GATOR1-related epilepsies. Somatic variants in *TSC1, TSC2* and *DEPDC5* have been detected in resected FCD tissue from patients with

germline mutations in matching mTOR genes, supporting the two-hit hypothesis[90, 91, 142, 144].

Somatic variants in genes within other signalling pathways also cause epileptogenic brain lesions. Brain mosaic variants in the galactose transporter gene *SLC35A2* cause mild MCD with oligodendroglial hyperplasia in epilepsy (MOGHE), a highly epileptogenic lesion with distinctive radiological features[145]. Somatic variants in genes encoding components of the Sonic hedgehog pathway are responsible for the development of hypothalamic hamartomas, which are midline brain lesions associated with gelastic (laughing) seizures. Mosaic somatic *GNAQ* variants cause Sturge-Weber syndrome. In this neurocutaneous disorder, leptomeningeal angiomatosis leads to refractory focal epilepsy[79]. Much of the hidden genetics in focal epilepsies may be explained by somatic variation in occult MCD, below the detection threshold of clinical MRI.

Despite the preponderance of ‘somatic’ lesional epilepsies, sequencing for germline variants still has diagnostic value in patients with MCD. In a Czech MCD cohort comprised of patients with FCD, HME, polymicrogyria, lissencephaly and periventricular nodular heterotopia, WES had a diagnostic yield of 21% (26/123)[146].

Table 1.4 Germline and somatic genetic causes of focal lesional epilepsies

Lesion	Germline mutations	Somatic mutations	References
FCD type I	*SCN1A*, *KCNT1*, *PCDH19*, *CNTNAP2*, *STXBP1* (rare case reports)	*SLC35A2*, *STXBP1*[a]	147-152
FCD type II	*TSC1*, *TSC2*, *DEPDC5*, *NPRL2*, *NPRL3, PTEN*	*TSC1*, *TSC2*, *MTOR*, *AKT3*, *PIK3CA*, *RHEB, DEPDC5*[a]	90, 127, 153-155
HME	*TSC1, TSC2, MTOR, DEPDC5, NPRL3*	*MTOR, TSC1, TSC2, AKT3, PIK3CA, PTEN*[a], *RHEB*	90, 91, 136, 156, 157
Bottom-of-sulcus dysplasia	*DEPDC5*, *NPRL3*	*MTOR*	158
Tuberous sclerosis complex	*TSC1, TSC2*	*TSC1, TSC2*[a]	136, 155
MOGHE	-	*SLC35A2*	159
Ganglioglioma	*NF1*	*BRAF* V600E	160, 161
DNET	*FGFR1, NF1*	*FGFR1*	161, 162
Periventricular nodular heterotopia	*FLNA, ARGEF2, MAP1B*, *NEDD4L,* 22q11.2 deletion	*FLNA*	163, 164
Lissencephaly	*DCX, LIS1, RELN, VLDLR, TUBA1A, TUBB2B, NDE1, DYNC1H1, ARX, CEP85L,* Miller-Dieker syndrome	*DCX, LIS1*	98, 164, 165
Polymicrogyria	*PTEN, PIK3R2, TUBA1A, TUBB2A, COL4A1/2, WDR62, NEDD4L*	1q trisomy, *PIK3CA, PIK3R2*	166-169
Cerebral cavernous malformations	*CCM1, CCM2, CCM3*	*PIK3CA, MAP3K3*, *AKT1, CCM1, CCM2, CCM3*[a]	170-172
Sturge-Weber Syndrome	-	*GNAQ*	173
Hypothalamic Hamartoma	*GLI3*	*GLI3, PRKACA, OFD1*	174, 175
Brain AVM	-	*KRAS*	176

Footnotes:
[a] Double-hit germline and somatic variants in *STXBP1, DEPDC5, TSC1, TSC2, PTEN, CCM1, CCM2* and *CCM3*

Abbreviations:
AVM= arteriovenous malformation; DNET= dysembryoplastic neuroepithelial tumour; FCD= focal cortical dysplasia; HME= hemimegalencephaly; MOGHE= mild malformation of cortical development with oligodendroglial hyperplasia

1.7 Precision medicine in the genetic epilepsies

Advances in genomic diagnostics have helped elucidate the genetic basis of many severe treatment-resistant epilepsies. Epilepsy gene discovery has augmented understanding of underlying disease mechanisms, providing targets for precision medicine. In 2011, the U.S. National Research Council published a report that advocated for a "*new taxonomy of human disease,*" introducing the concept of "precision medicine"[177]. The field of oncology was the first to embrace large-scale precision medicine approaches. “Precision oncology” has entered mainstream clinical practice, with increasing use of genomic tumour profiling to guide cancer therapies. Adoption of precision medicine in the epilepsies has been more elusive, owing to the complexity of underlying biological mechanisms. Disease mechanisms underlying monogenic epilepsies include: (a) ion channel defects, (b) synaptic machinery dysfunction, (c) transporter and enzyme-mediated disruptions in metabolic pathways, (d) mTOR pathway dysregulation, and (e) disorders of transcription, DNA repair and chromatin remodelling[178]. A spectrum of increasing therapeutic precision can be applied to monogenic epilepsies. Byrne and colleagues classified precision treatments for monogenic epilepsies into six tiers based on how precisely they target the underlying aetiology (see **Figure 1.7**)[179].

Tier 1 treatments comprise ASMs with superior efficacy in specific epilepsy phenotypes. Examples include sodium valproate for IGE and rufinamide for Lennox-Gastaut syndrome. Tier 1 treatments target specific seizure types within syndromes, and genetic factors are unlikely to be relevant. Tier 2 treatments are ASMs that perform better in specific monogenic epilepsies, but the mechanism of action in relation to the gene is poorly understood. Examples include carbamazepine for *PRRT2*-related epilepsy and levetiracetam for *PCDH19* female-limited epilepsy[180, 181]. These examples embody “*stratified*” or “*personalised*” medicine, as the ASMs do not target underlying disease-specific mechanisms.

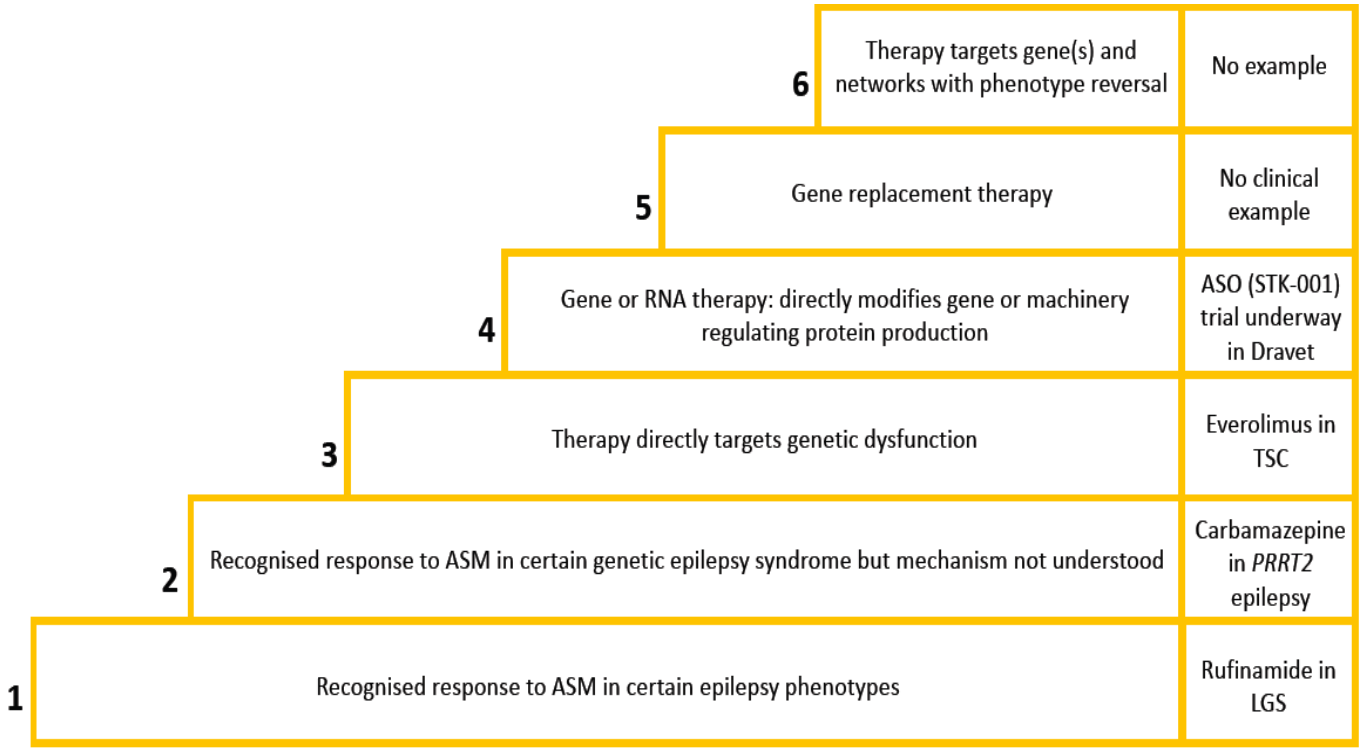

Figure 1.7 Tiers of precision therapy in the epilepsies

Figure used with permission from Byrne, Enright and Delanty, 2021[179].

Abbreviations: ASM= antiseizure medication; ASO= antisense oligonucleotide; LGS= Lennox-Gastaut syndrome; TSC= tuberous sclerosis complex.

Tier 3 consists of precision treatments that aim to modulate or bypass the dysfunction caused by the mutated gene product[179]. Tier 3 treatments are used successfully in channelopathies, mTORopathies, and epilepsies caused by genetic alterations in metabolic pathways. Seizures in channelopathies are caused by altered neuronal excitability due to increased (GoF) or decreased (LoF) channel currents. Careful functional characterisation of channel currents can enable strategic use of existing ASMs or repurposed drugs to treat seizures. Different variants within the same gene can exert GoF or LoF effects, leading to distinctive phenotypes and opposite treatment implications. For example, GoF variants in *SCN2A* cause infantile onset self-limiting focal epilepsy or DEE that responds well to sodium channel blockers. In contrast, LoF variants in *SCN2A* cause DEE presenting later in childhood, without meaningful response to sodium channel blockers[182]. Pathogenic variants in *KCNA2,* encoding a voltage-gated potassium channel, cause severe DEE through GoF effects on channel current. Precision treatment with repurposed 4-aminopyridine, a voltage-gated potassium channel blocker used in multiple sclerosis and Lambert-Eaton Myasthenic syndrome, improved seizure control in patients with pathogenic variants in *KCNA2*[183].

Tier 3 also includes treatments that replace what is missing, or bypass relevant pathway steps in metabolic genetic epilepsies. As previously discussed, the ketogenic diet provides an alternative energy source to the brain in GLUT1-deficiency syndrome[8]. Biallelic variants in *CCDS* cause guanidinoacetate methyltransferase (GAMT) deficiency. The clinical phenotype consists of ID, epilepsy, ASD and movement disorders. The enzymatic defect leads to cerebral creatine deficiency. Early treatment with creatine supplementation can improve cognitive and seizure outcomes[184]. Epilepsy in TSC and other mTORopathies is caused by hyperactivation of the mTOR pathway. Everolimus, a synthetic mTOR inhibitor, is a licensed precision therapy for TSC-related DRE.

Precision treatments in tier 4 are not yet clinically available for genetic epilepsies, but are expected to emerge in the not too distant future. Tier 4 treatments aim to improve phenotype by restoring function (at least partially) of the mutated gene. Current knowledge suggests that modulation of gene expression through ribonucleic acid (RNA) binding is the most accessible mechanism to achieve this target. Gene

expression can be regulated using antisense oligonucleotides (ASO) by targeting RNA for degradation, preventing the translation of a specific RNA into a protein, and altering the splicing of pre-messenger RNA (mRNA). ASOs are synthetic, short (18-30 base pairs), single-stranded DNA or RNA sequences that bind to target RNA through 'Watson-Crick' base pairing[185]. ASO precision treatments are already in use in several neurological conditions, including spinal muscular atrophy, Duchenne muscular dystrophy and transthyretin familial amyloid polyneuropathy[186]. ASOs can increase production of mRNA by modulating splicing in a technique known as "targeted augmentation of nuclear gene output" (TANGO). Seizures and mortality were reduced in a Dravet syndrome mouse model using the TANGO approach[187]. A TANGO-based treatment is now undergoing phase 1 and 2 clinical trials in human epilepsies caused by LoF *SCN1A* variants[178]. Alternatively, ASOs that reduce gene expression by inhibiting transcription of mRNA could be used to treat monogenic epilepsies caused by GoF variants (for example, *SCN8A*-related DEE).

An individualised ASO (Milasen) was developed for a child with neuronal ceroid lipofuscinosis 7, a lethal neurodegenerative condition that causes visual loss, ataxia, seizures and cognitive impairment. Milasen decreased seizure burden and appeared to stabilise neurological function[188]. Although the patient ultimately died, the Milasen story illustrates that it is possible to rapidly design, test and deploy a novel ASO therapy that targets a unique pathogenic variant. The case also raised ethical issues pertaining to equity, as a foundation run by the patient's mother funded the development and administration of Milasen[189].

Tier 5 and 6 precision treatment approaches for epilepsy are currently speculative. Treatments in tier 5 involve replacing the mutated gene with a normal functioning copy or transgene[179]. This approach requires efficient delivery of the gene therapy to target tissue or cells through vehicles called vectors. Adeno-associated virus vectors (AAV) are currently one of the leading platforms for gene therapy. AAVs are small, nonenveloped viruses belonging to the *Parvoviridae* family and have tissue-specific tropism determined by their capsids, allowing for tissue-specific transduction[190]. For example, the AAV9 serotype allows for central nervous system (CNS) transduction. Recombinant AAVs are generally considered non-pathogenic in humans, as they lack the ability to replicate in human hosts. Additionally, the risk of insertional mutagenesis,

where the introduction of foreign DNA may cause mutations in the host genome, is considered low, as AAVs mostly remain as extrachromosomal episomes[190]. Current AAV-based techniques are limited by the amount of DNA they can cargo (genes like *SCN1A* are too large) and difficulties controlling gene dosing.

AAVs have superseded previously used adenoviral platforms due to their potential for long-lasting gene expression (a single administration can permanently alter gene expression), coupled with reduced immunogenicity. However, adverse immunological events have been observed in clinical trials, including immune haemolysis thrombocytopenia, thrombotic microangiopathy and immune-mediated kidney injury[190]. Host immune responses can be directed against AAV capsid proteins or transgene-encoded products[191]. Immunomodulatory strategies, including corticosteroid prophylaxis, are commonly used to mitigate immune-mediated adverse events. Onasemnogene abeparvocec is a licenced AAV-based gene therapy for spinal muscular atrophy. An AAV-based approach was used in a preclinical model of focal epilepsy to reduce neuronal excitability through overexpression of a potassium channel gene[192]. However, AAV-based gene therapies have not yet been used in human epilepsy patients. It is anticipated that an AAV-based gene therapy for epilepsy may require direct intracerebral administration, which may pose associated neurosurgical risks.

AAVs can also function as vectors for ASOs and clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 systems[178]. CRISPR-Cas9 genome editing was adapted from a naturally occurring bacterial anti-viral defence system. Using this approach, Cas9 enzyme is directed to a genomic region by a synthetic guide RNA, where it can cut or insert DNA, to inactivate mutated genes. A gene editing approach that has shown promise in a mouse model of Dravet syndrome, involves mutated “dead” Cas9 (dCas9). Instead of cutting DNA, dCas9 fuses with gene transcription regulators with the potential to activate or repress gene expression. This approach was used to upregulate *Scn1a* expression in GABAergic inhibitory neurons in a mouse model of Dravet syndrome, leading to reduced seizures and improved behaviour[193]. Clinical translation of CRISPR-Cas9 editing has been hampered by varying efficiency, off-target effects and insufficient vector size. Furthermore, introduction of “foreign” Cas9 enzyme brings risks related to immunogenicity[178]. The tier 6 approach

represents the ideal precision treatment, that would target the gene(s) and networks involved in a genetic epilepsy, imparting freedom from seizures and co-morbidities[179].

Recent developments with ASOs and other genetic therapy approaches have brought us closer to the precision medicine ideal. However, a recent survey of 293 patients with monogenic epilepsies found that only 11% were treated with precision medicines, and less than 5% benefited from treatment, demonstrating that effective precision therapies do not exist for most monogenic epilepsies[194]. Moreover, with the exception of everolimus in TSC, all evidence for treatments described in previous paragraphs are derived from single case reports or small case series. Rigorous testing in sophisticated preclinical models and innovative clinical trial designs are required to successfully implement novel precision therapies in monogenic epilepsies.

Several preclinical models are available to study disease mechanisms and test precision therapies. Human genetic material can be expressed heterologously in cell lines including *xenopus oocytes* and human embryonic kidney 293. Patient-derived induced pluripotent stem cells (iPSCs) can differentiate into any cell type, including neurons. Neurons derived from iPSCs were used to study *SCN8A* GoF variants, identifying riluzole as a potential precision treatment[195]. Patient-derived iPSCs can self-organise into three-dimensional spheroids in culture (cerebral organoids), with better recapitulation of the structural features and cellular heterogeneity of human brains. Zebrafish models of monogenic epilepsies are amenable to high-throughput drug screens, due to rapid breeding cycles, low space requirements, ease of drug administration into water environments, and the ability to automate seizure recordings[189].

Natural history studies that quantify the dynamic phenotypes of monogenic epilepsies will inform the design of gene-specific outcome measures for clinical trials, including cognitive and neuropsychiatric endpoints. Given the rarity of most monogenic epilepsies, innovative trial designs for small sample sizes are needed to increase rigor and generalisability. The "*n-of-1*" multi-crossover trial design has been proposed as a more robust tool to study precision treatments in rare genetic epilepsies. The main principle of the "*n-of-1*" design is that the participant serves as their own control via sequential treatment phases of active drug, and then placebo[196]. In addition, "*n-of-*

some” clinical networks of trial ready patients with functionally characterised variants in specific genes may facilitate precision therapy trials.

Table 1.5 Examples of precision treatments for monogenic epilepsies

Disease mechanism	Disease genes	Tiers 2 treatments	Tier 3 treatments	Tier 4-6 treatments
Ion channel/ receptor dysfunction	*SCN1A/2A/8A, KCNQ2, KCNT1, KCNA2, CACNA1A, CHRNA4/2, GRIN2A/B*	Stiripentol, fenfluramine and CBD (*SCN1A* LoF); Acetazolamide *(CACNA1A* DEE)	SCBs (*SCN1A/2A/8A* GoF); 4-AP (*KCNA2* GoF)*;* retigabine in (*KCNQ2* LoF); nicotine (*CHRNA4* GoF); memantine (*GRIN2A/B* GoF)	STK-001 (ASO) phase 1 and 2 clinical trials *SCN1A* LoF
Synaptic machinery dysfunction	*PRRT2, STXBP1, STX1B, VAMP2, SNAP25, DNM1, DOCK7, SYNGAP1*	Carbamazepine (*PRRT2)*	Nil	Nil
Transporter failure in a metabolic pathway	*SLC2A1 (*GLUT1), *SLC19A3 (*thiamine), *SLC6A1 (*GABA), *SLC35A2 (*galactose)	Valproate (*SLC6A1*-related myoclonic-atonic epilepsy)	Ketogenic diet *(SLC2A1);* biotin and thiamine (*SLC19A3);* galactose (*SLC35A2* somatic and germline)	Nil
Enzymatic failure in metabolic pathway	*ALDH4A1/7A1, PNPO, BTD, FOLR1, GAMT, ATP7A, CAD*	Nil	Pyridoxine (*PNPO, ALDH4A1/7A1);* biotin (*BTD);* folinic acid (*FOLR1);* creatine (*GAMT);* copper (*ATP7A);* uridine (*CAD)*	Nil
mTOR pathway dysregulation	*TSC1, TSC2, DEPDC5, NPRL2/3, STRADA*	CBD and vigabatrin (*TSC1/2)*	Everolimus (*TSC1/2)*	Nil
Disorders of DNA repair, transcription regulation, chromatin remodelling	*CDKL5, ARX, CHD2, FOXG1, MECP2*	Ganaxolone and fenfluramine (*CDKL5)*	Nil	Nil

Abbreviations:
ASO= antisense oligonucleotide; CBD= cannabidiol; DEE= developmental and epileptic encephalopathy; GLUT1= glucose transporter 1; GoF= gain-of-function; LoF= loss-of-function; SCB= sodium channel blocker; 4-AP= 4-Aminopyridine.

1.7.1 Epilepsy in the mTORopathies: opportunities for precision medicine

Some epilepsy genes converge on shared homeostatic pathways, like the mTOR cascade. Systems biology approaches decode molecular and biochemical interactions within complex biological networks, to understand “larger picture” downstream consequences[189]. Applying a systems biology approach to understand the functional effects of different epilepsy-associated mTOR pathway mutations consistently identifies mTOR pathway hyperactivation as a shared downstream consequence. Treatments that target mTORC1 hyperactivity in TSC reduce seizure frequency and tumour size.

The mTOR signalling cascade serves as a ubiquitous regulator of cell metabolism, growth, proliferation and survival. In the nervous system, the mTOR pathway has important functions related to synaptic transmission and plasticity, neural network activity, and neurogenesis[128, 197]. Pathogenic variants in genes encoding proteins within the mTOR cascade cause epilepsies , MCD and neurodevelopmental disorders. The neurological manifestations of TSC include epilepsy, FCD, subependymal giant-cell astrocytoma (SEGA) and neuropsychiatric disorders. Brain somatic variants in mTOR pathway genes cause FCD type II and HME. The GATOR1 complex directly inhibits mTOR activity in response to intracellular amino acid levels[128]. Pathogenic variants in genes encoding the GATOR1 complex cause non-lesional and FCD-related epilepsies, that are often treatment-resistant. The anti-seizure effects of mTOR inhibitors seen in TSC may also be applicable to other mTORopathies, as mTOR pathway hyperactivation is a shared pathomechanism[136].

1.8 Thesis outline and aims

This thesis emerged out of efforts to incorporate a precision medicine approach to the management of epilepsies caused by mTOR pathway genetic variants. Collectively, the mTORopathies are characterised by excessive mTOR pathway activation and a high propensity for developing DRE. Chapter 2 outlines the common methodologies used throughout the thesis. Chapter 3 reviews the literature on the molecular disease mechanisms underlying epilepsy in the mTORopathies. In order to better understand the potential impact of precision therapies in mTORopathies, chapter 3 outlines the spectrum of epilepsies caused by mutations in mTOR pathway genes.

Chapter 4 reviews the clinical pharmacology of currently available mTOR inhibitors and evaluates their safety and efficacy for treatment of neurological manifestations in mTORopathies. As this project began during the pandemic, chapter 4 concludes with a review of safety data on mTOR inhibitor use in the context of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. Chapter 5 presents a retrospective analysis of everolimus efficacy and safety in a cohort of adult TSC patients with DRE attending Irish epilepsy clinics.

Chapter 6 begins with a literature review on the epidemiological, genetic and phenotypic characteristics of GATOR1-related epilepsies. This is followed by a “deep phenotyping" analysis of patients with GATOR1-related epilepsies identified through genomic diagnostic research at the FutureNeuro Research Centre. Chapter 7 examines whether everolimus precision treatment improves seizure outcomes in patients with refractory GATOR1-related epilepsies, in an *“n-of-some”* study.

Chapter 8 reviews the value of incorporating genetic testing in the epilepsy surgery evaluation. This chapter reviews whether focal epilepsies caused by mTOR gene mutations have favourable epilepsy surgery outcomes, compared with other monogenic epilepsies.

Thesis aims:

a) To delineate the clinical and genetic characteristics of epilepsies caused by mTOR pathway genetic variants, with particular emphasis on the GATOR1-related epilepsies
b) To establish dosing and monitoring protocols for everolimus treatment in mTORopathies
c) To study the safety and efficacy of everolimus treatment for DRE in an adult TSC cohort
d) To explore the potential of everolimus treatment for refractory GATOR1-related epilepsies
e) To outline a precision medicine framework for the management of epilepsies in mTORopathies, including an epilepsy surgery consideration

2. Common Methodology

To examine the potential impact of applying a precision medicine approach for the management of epilepsy in mTORopathies, three literature-based studies, one phenotyping study, and two observational studies of everolimus treatment are presented. The three literature reviews summate and analyse available data regarding three related topics:

a) The clinical and genetic spectrum of mTOR-related epilepsies
b) The clinical pharmacology of mTOR inhibitors, and rationale for use in non-TSC mTORopathies
c) The utility of genetic testing in the epilepsy surgery evaluation

In addition to this section outlining the common methodology of the thesis, each subsequent chapter will contain a section describing detailed methodology specific to its content. Chapters 3, 4 and 8 present the literature-based studies. Chapter 5 reports a retrospective cohort study of adult TSC patients treated with everolimus for DRE. Chapter 6 describes a case series of patients with GATOR1-related epilepsies. Chapter 7 reports a prospective study of everolimus precision therapy for refractory GATOR1-related epilepsies. The findings reported in Chapters 6 and 7 are the product of genomic research carried out at the FutureNeuro Research Centre in the Royal College of Surgeons in Ireland (RCSI), Beaumont Hospital, and Children's Heath Ireland (CHI) at Temple Street.

2.1 Ethical approval

Ethical approval is in place for a biobank of DNA and associated clinical details from epilepsy patients attending Beaumont Hospital (REC 14/44, version 4). The "*Genetics of Epilepsy*" biobank was initiated in 2002 to improve our understanding of how genes influence the cause and development of epilepsy, as well as increasing knowledge on how epilepsy patients respond to treatments. Clinical data is stored and maintained in pseudonymised form on a password encrypted study database managed by the RCSI biobanking service, based at the RCSI Beaumont campus. Linked genetic data is stored at RCSI on the RCSI Research Information Technology Compute Cluster. This

cluster is designed for storing and processing personal data, and has extensive industry-standard security hardening applied to the servers in support of this. My supervisor Professor Norman Delanty initiated the Irish epilepsy biobank, who together with my other supervisor Professor Gianpiero Cavalleri have used this rich source of clinical and genetic data to answer many important research questions.

The prospective everolimus study for patients with GATOR1-related DRE was approved by the Beaumont Hospital (Medical Research) Ethics Committee (REC 21/33) (see **Appendix 1**). Written, informed consent was obtained from all adult participants with decision-making capacity (see **Appendix 2** for information sheets and consent forms). A consent declaration was obtained from the Irish Health Research Consent Declaration Committee (HRCDC) for adults lacking decision-making capacity (see **Appendix 3**). Parents provided written informed consent for participants younger than 18-years-old (see **Appendix 2C and 2F**). Participants and proxies were consented for off-label use of everolimus.

The study of everolimus in patients with TSC did not require ethics committee review or patient consent, as it was a retrospective analysis of an approved treatment using existing clinical data.

2.2 The Epilepsy Electronic Patient Record

Much of the clinical data described in this thesis was extracted from the Beaumont Hospital epilepsy Electronic Patient Record (EPR). The epilepsy EPR is a secure web-based electronic health record that was implemented in Beaumont Hospital in 2009, and is now operative in several Irish hospitals. The initiative was led by Professor Delanty and Ms Mary Fitzsimons, a medical physicist who has been instrumental at developing epilepsy eHealth infrastructure in Ireland. The EPR was designed to include epilepsy-specific data modules. Bespoke modules include: epilepsy history and aetiological factors; seizure types and frequency; current and previous ASMs; VNS and epilepsy surgery data; co-morbidities; clinical investigation results; clinic visit outcomes and epilepsy specialist nurse advice line interactions. Dynamic data such as ASM changes and seizure frequencies can be tracked over time to monitor for treatment responses.

A ‘genomics module’ is the latest addition to the EPR, including genomic data (candidate genetic variants with ACMG classification) and additional phenotypic data (pedigree and clinical photography for dysmorphology assessments)[198]. The genomics module facilitates discussion at the epilepsy genetics MDT review meeting which is described in greater detail in **section 2.6**. Access to the EPR is role-based, in that authorised personal can only access EPR modules relevant to their clinical or research roles. For example, only clinicians and researchers that participate in the epilepsy genetics MDT meeting have access to the genomics module.

The EPR was designed to improve standardisation of epilepsy-specific terminology in medical records, to support delivery of clinical services and to assist research and health services monitoring. A reporting tool facilitates efficient retrieval and analysis of data on predefined patient populations[199]. Several studies have shown that the epilepsy EPR functions in a meaningful way, supporting clinical care, monitoring service quality and providing rich clinical data for research[76, 199, 200]. The epilepsy nurse specialists have been crucial to the success of the EPR, as they populate the database with new patient clinical data. With assistance from Mr. Roger Grogan, the National Epilepsy System Technical Lead, I extracted clinical data from the EPR for analysis in this thesis.

2.3 Next-generation sequencing

Most patients with GATOR1-related epilepsies were identified via the genomics research programme at the FutureNeuro Research Centre in RCSI. This research could not have been carried out without the expertise of Dr. Katherine Benson, a postdoctoral genomics researcher and bioinformatician at FutureNeuro and RCSI. Dr Benson provided adept interpretation of all sequencing data obtained as part of this study, and guided the bioinformatics procedures of FutureNeuro’s broader genomic diagnostic research programme. This methodology is described by Dr. Benson in a study examining the diagnostic yield of WES in epilepsy with co-morbid ID, undertaken at the RCSI FutureNeuro research centre[10].

DNA samples from probands and their parents (if available) were extracted from peripheral blood lymphocytes, saliva or buccal swabs using Qiagen’s QIAcube technology in a research setting. A second DNA sample was extracted in an

accredited laboratory setting (CeGaT GmbH, Germany) for confirmatory DNA testing, as required. WES was conducted using Roche NimbleGen SeqCap EZ V3, as per the manufacturer's instructions using 500ng of input gDNA. Sequencing was performed on an Illumina NextSeq platform. Targeted panel sequencing was performed using a custom Roche NimbleGen SeqCap panel (see **Table 2.1** for list of genes on epilepsy panel) as per manufacturer's instructions using 500 ng of input DNA and sequenced using an Illumina MiSeq.

Table 2.1 List of targeted genes in epilepsy panel (166 genes)

ABAT, ADSL, ALDH1A3, ALDH5A1, ALDH7A1, ALG13, AMT, ARHGEF9, ARX, ATP1A2, ATP1A3, ATP6AP2, ATRX, CACNA1A, CACNA1H, CACNB4, CASK, CASR, CCM2, CDKL5, CHD2, CHRNA2, CHRNA4, CHRNB2, CLCN2, CLN3, CLN5, CLN6, CLN8, CNTNAP2, CPA6, CPT2, CSTB, CTSD, CUL4B, DCX, ***DEPDC5****, DNAJC5, DNM1, DNM1L, DYRK1A, EFHC1, EMX2, EPM2A, FLNA, FOLR1, FOXG1, GABRA1, GABRB3, GABRD, GABRG2, GAMT, GATM, GCSH, GNAO1, GNB1, GOSR2, GPHN, GRIA3, GRIN1, GRIN2A, GRIN2B, HNRNPU, HSD17B10, IQSEC2, KCNA1, KCNA2, KCNB1, KCNJ10, KCNJ11, KCNMA1, KCNQ2, KCNQ3, KCNT1, KCTD7, KDM5C, KDM6A, KRIT1, LGI1, MAGI2, MBD5, ME2, MECP2, MEF2C, MFSD8, MOCS1, MOCS2, MTHFR,* ***MTOR****, NEDD4L, NHLRC1,* ***NPRL2, NPRL3,*** *NRXN1, OFD1, OPHN1, PAFAH1B1, PAK3, PCDH19, PDCD10, PGK1, PHF6, PIGV,* ***PIK3R4****, PLCB1, PNKP, PNPO, POLG, PPT1, PRICKLE1, PRICKLE2, PRRT2, RAB39B, RAI1, RELN, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, SCARB2, SCN1A, SCN1B, SCN2A, SCN4A, SCN5A, SCN8A, SCN9A, SERPINI1, SIK1, SLC1A3, SLC19A3, SLC25A22, SLC2A1, SLC35A2, SLC6A1, SLC6A8, SLC9A6, SMC1A, SMS, SPTAN1, SRPX2, ST3GAL3, ST3GAL5,* ***STRADA****, STX1B, STXBP1, SYN1, SYNGAP1, SYP,* ***SZT2****, TBC1D24, TCF4, TPP1, TREX1,* ***TSC1****,* ***TSC2****, TUBA1A, TUBA8, TUBB2A, TUBB2B, TUBB3, TUBB5, TUBG1, UBE3A, WDR62, ZEB2*

Footnotes:

mTOR pathway genes are highlighted in bold font.

2.4 Bioinformatic analyses

Exome data was aligned to the GRCh37/hg19 reference genome[201] and processed using a Burrows-Wheeler Aligner and Picard[202]. Variants were identified using the Genome Analysis ToolKit (GATK) best practices protocol and annotated using ANNOVAR. Exomes with a minimum of 85% of target bases covered at a minimum depth of 10x were included for analysis. The relatedness of probands and parents were confirmed using identity by descent testing in PLINK[203]. Bioinformatic pipelines were hosted on a dedicated Microsoft Azure server. Following analysis using the inhouse pipeline, unsolved cases were analysed using a separate pipeline via SapientiaTM v1.9, a commercially available diagnostic decision support platform by Congenica Ltd (Hinxton, UK).

2.5 Variant classification

Candidate variants from exome sequencing were selected for discussion at the epilepsy genetics MDT meeting if they satisfied the following criteria:

a) Minor allele frequency (MAF) of <1% for variants with recessive inheritance patterns, or MAF of <0.1% for variants with dominant inheritance patterns in gnomAD control database[204].
b) Variant predicted to be damaging by at least two of three prediction software tools: PolyPhen[205], SIFT[206] and/or MutationTaster[207].
c) Variant in gene known to cause either epilepsy or ID (as per the Online Mendelian Inheritance in Man (OMIM, https://omim.org/[208]) or included in the ACMG recommended list of clinically actionable incidental findings[209].

Candidate genes from across the exome were assessed for an epilepsy and/or ID phenotype using OMIM for each case. Variants assessed were not limited to the gene panel. CNVs were considered if:

a) Large (>1 Mb).
b) Spanned a gene listed in OMIM as morbid in neurological disease.
c) Not commonly seen in control populations (<3 similar sized variants in non-disease populations in the Database of Genomic Variation)[210].

Genomic results were uploaded to the epilepsy EPR using the bespoke genomics module[198]. The genomics module enables the integration of clinical and genomics data, discussion of each patient's genomics results at the MDT meeting, as well as sharing of clinically relevant results with referring clinicians.

2.6 The epilepsy genetics multidisciplinary team meeting

Candidate variants were discussed at the epilepsy genetics MDT meeting, with input from a clinical geneticist (Dr. Marie Greally), geneticists/bioinformaticians (Dr. Katherine Benson, Prof. Gianpiero Cavalleri), adult and paediatric neurologists (Prof. Norman Delanty, Prof. Daniel Costello, Prof. Colin Doherty, Dr. Susan Byrne, Dr. Hugh Kearney), epilepsy genetics research fellows (Dr. Patrick Moloney and Dr. Michael Doyle) and an epilepsy advanced nurse practitioner (Maire White). The MDT meeting is held every second month for review of candidate variants identified via research WES or WGS, as well as being a forum to discuss diagnostically challenging cases (often VUSs) identified through clinical genetic testing. Recently, the MDT established a virtual national genomics review meeting to assist neurologists and trainees in Ireland with interpretation of genetic variants in neurology patients.

The MDT considers the inheritance pattern of candidate genomic variants. If multiple first-degree relatives were affected, the relevant variant was tested for segregation in additional family members. Variants that did not match the expected segregation pattern for pathogenicity, were considered benign, or as VUSs.

2.7 Clinical interpretation and variant validation

Candidate variants were classified using ACMG guidelines[93]. Cascade testing was considered when requested by family members. Candidate variants were confirmed on an independent DNA sample using Sanger sequencing, array-CGH or MLPA as appropriate. Confirmation testing was conducted by CeGaT GmbH, Germany. CeGaT also provided an independent clinical genetics interpretation of variant pathogenicity. Once the presence and pathogenicity of candidate variants were confirmed by CeGaT, results were returned to the patient by the treating clinician. All variants discussed in this thesis have been submitted to *ClinVar.*

2.8 Incidental findings

During the consent process, all adult patients were informed about the possibility of incidental findings and given the option to receive results on "*clinically actionable*" incidental findings, as previously defined by the ACMG[209, 211]. Clinically actionable genetic variants are those associated with disorders for which preventative measures or treatments are available.

3. The clinical and molecular spectrum of mTOR-related epilepsies

3.1 Introduction

Both germline and somatic variants in genes encoding for different components of the mTOR signalling cascade cause epilepsies, MCD and neurodevelopmental disorders, collectively referred to as mTORopathies[136]. A systems biology approach identifies neuronal mTOR pathway hyperactivation as a driver of seizures and other neurological manifestations in all mTORopathies. Knowledge of the "normal" physiological function of mTOR pathway regulators, provides a framework for understanding how mutated genes encoding pathway regulators culminate in mTOR hyperactivity and seizures.

3.1.1 The mTOR cascade

The mTOR-signalling cascade serves to maintain cellular homeostasis and energy metabolism in response to diverse cellular and environmental stimuli. The mTOR pathway modulates cell proliferation and growth, protein synthesis, transcription, autophagy, and organelle biogenesis and maintenance[212]. The mTOR cascade has important functions in the brain related to synaptic transmission and plasticity, neural network activity and neurogenesis[128].

The serine/threonine protein kinase mTOR is ubiquitously expressed, with particularly high levels in the brain. It forms part of two functionally distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is a central signalling node, receiving inputs from upstream regulatory proteins that are influenced by growth factors (for example, insulin), adenosine triphosphate (ATP) concentrations and nutrients (for example, amino acids). When activated, mTORC1 promotes cell growth and survival via regulation of mRNA translation, nucleotide biosynthesis and cellular autophagy. Downstream substrates of mTORC1 signalling that modulate these pivotal cellular processes include ribosome S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein. The activity of mTORC1 is mainly regulated by three converging signalling pathways: (a) the growth factor pathway, (b) the

energy/ATP-sensing pathway, and (c) the amino acid-sensing pathway (see **Figure 3.1**)[128, 212, 213].

Rapamycin (also known as sirolimus) is produced by the bacterium *Streptomyces hygroscopius* and is an inhibitor of mTORC1 signalling. Rapamycin inhibits mTORC1 activity by forming a complex with FK506 binding protein 1 A 12 kDa (FKBP12). The FKBP12-rapamycin binding complex interacts with mTOR and inhibits mTORC1 by an allosteric mechanism[128]. mTORC2 is primarily involved in cytoskeletal integrity and cell migration. mTORC2 is insensitive to rapamycin inhibition[214].

TSC1 and TSC2 proteins (also known as hamartin and tuberin, respectively) are part of a heterotrimeric complex with Tre2-Bub2-Cdc16 1 domain family member 7 (TBC1D7), known as the TSC protein complex. The TSC protein complex indirectly inhibits mTORC1 via Ras homologue enriched in brain (Rheb), which is the target of the GTPase-activating domain of TSC2[212, 213]. TSC2 is a common actor in the growth factor-responsive and the ATP-sensing arms of the mTOR signalling pathway[128].

Growth factors, such as insulin and insulin-like growth factor-1 stimulate phosphoinositide 3-kinase (PI3K) to trigger phosphoinositide-dependent kinase 1 (PDK1) to phosphorylate and activate Akt (also known as protein kinase B [PKB]). TSC2 activity is repressed by Akt-mediated phosphorylation, which releases the TSC protein complex's inhibitory effect on mTORC1 signalling. Phosphatase and tensin homologue (PTEN) protein directly inhibits PI3K-Akt pathway signalling[212, 213].

The STE20-related kinase adaptor alpha (STRADα) and liver kinase B (LKB) complex is an upstream regulator of the energy-sensing arm of the mTOR pathway[128]. In response to depleted ATP, the STRADα/ LKB complex inhibits mTORC1 signalling by activating TSC2 via phosphorylation of adenosine monophosphate-activated kinase (AMPK)[215].

GATOR1 complex is the principal amino acid-sensing regulator of mTORC1 signalling. GATOR1 complex is composed of three subunits: DEPDC5, NPRL2 and NPRL3. GATOR2 complex inhibits GATOR1 in response to increasing amino acid levels, resulting in mTORC1 disinhibition, facilitating pathways for cell growth. Leucine,

arginine and methionine are potent activators of mTORC1 signalling via GATOR1 and GATOR2 complexes[216]. When amino acid levels are low, GATOR1 directly inhibits mTORC1 activity[217]. Caloric restriction and acute fasting reduce mTORC1 signalling through DEPDC5-mediated mechanisms[218]. Kaptin (KPTN), integrin alpha FG-GAP repeat containing 2 (ITFG2), chromosome 12 open reading frame 66 (C12orf66) and seizure threshold 2 (SZT2) form the KICSTOR complex (KPTN, ITFG2, C12orf66 and SZT2-containing regulator of mTORC1). The KICSTOR complex scaffolds GATOR1 to the lysosomal surface[219].

Using the information presented in the previous paragraphs, it stands to reason that LoF mutations in pathway inhibitors (*TSC1, TSC2, DEPDC5, NPRL3, NPRL2, PTEN, STRADA*) and GoF mutations in pathway activators (*PI3KCA, AKT3, RHEB, MTOR*) culminate in hyperactivation of mTORC1.

The aims of this chapter are:

a) To systematically analyse the epilepsy syndromes associated with pathogenic variants in genes encoding different mTOR pathway regulators.
b) To evaluate the evidence supporting mTORC1 hyperactivation as an essential pathomechanism of epilepsy in mTORopathies.

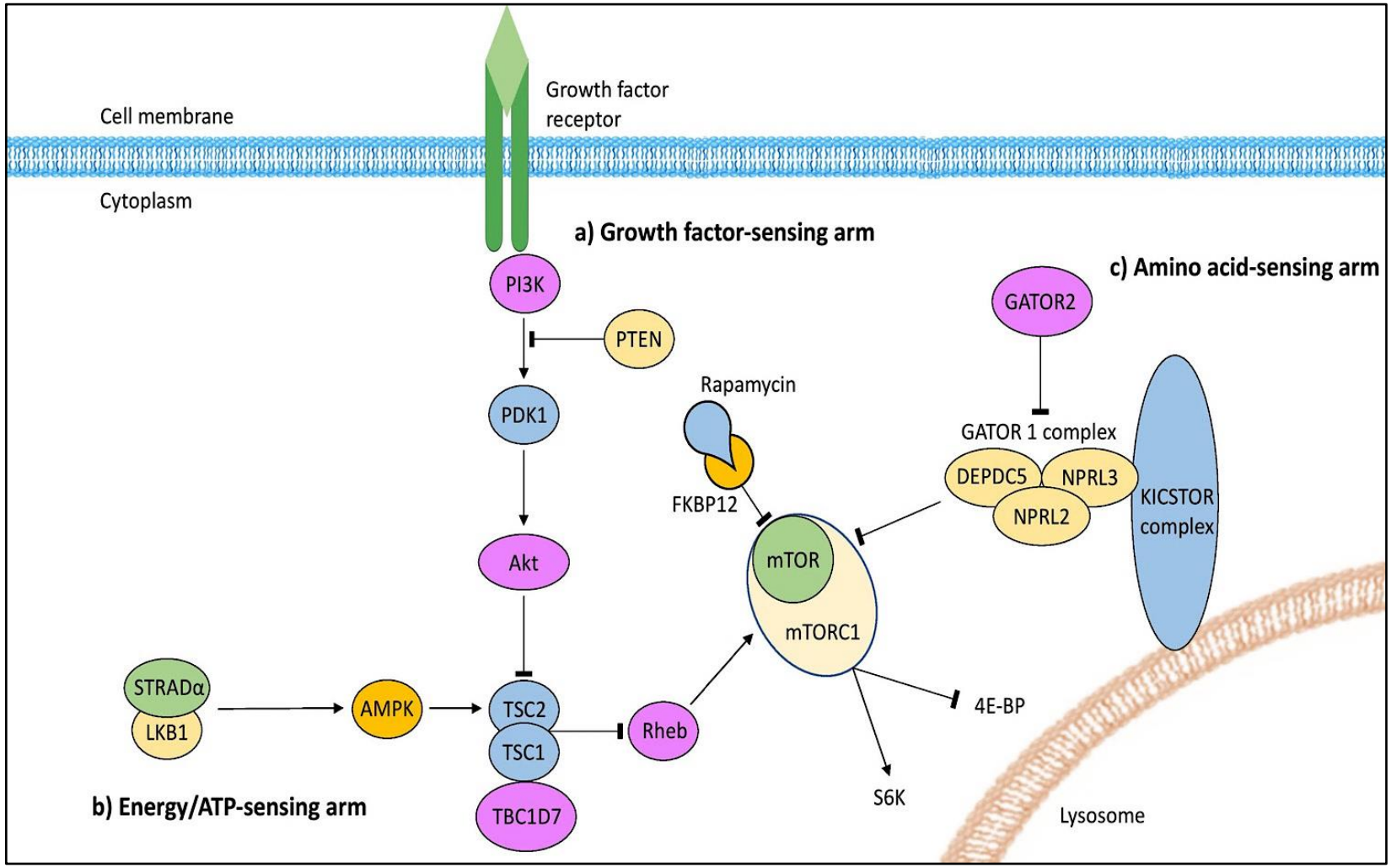

Figure 3.1 The mTOR cascade and its regulators.

Figure is taken from Moloney, Cavalleri and Delanty, 2021[136].

Abbreviations: AMPK= adenosine monophosphate-activated kinase; DEPDC5= dishevelled, Egl-10, and Pleckstrin domain-containing protein 5; FKBP12= FK506 binding protein 1 A 12 kDa; GATOR= GAP activity towards Rags; KICSTOR= KPTN, ITFG2, C12orf66 and SZT2-containing regulator of mTORC1; LKB= liver kinase B; mTOR= mechanistic target of rapamycin; mTORC1= mTOR complex 1; NPRL2/3= nitrogen permease regulator-like 2/3; PDK1= phosphoinositide-dependent kinase 1; PI3K= phosphoinositide 3-kinase; PTEN= phosphatase and tensin homologue; STRADα= STE20-related kinase adaptor alpha; S6K= ribosomal S6 kinase; TBC1D7= TBC1 domain family member 7; TSC1/2= tuberous sclerosis complex 1/2; 4E-BP= 4E-binding protein-1.

3.2 Methods

3.2.1 Delineating the clinical and molecular spectrum of mTOR-related epilepsies

Epilepsy-associated mTOR genes were identified through four methods:

a) Personal experience of encountering patients with genetic epilepsy and reading academic literature describing cases.
b) Review of genes covered by epilepsy diagnostic testing in UK National Health Service laboratories and German commercial laboratories (CeGat and Centogene).
c) Search of the OMIM resource using the term [epilepsy][208]. *Search date November 2nd, 2022; 1396 results*.
d) Search of PubMed [1993-2022] using the search terms ([epilepsy] OR [seizure]) AND ([mTOR] OR [mechanistic target of rapamycin] OR [mammalian target of rapamycin]) AND ([gene] OR [genetic]). *Search date November 14th, 2022; 752 results*.

For each epilepsy-associated mTOR pathway gene identified I reviewed the original research for quality and relevance. Any genes considered relevant to epilepsy were added to a database, in which I recorded phenotype features (seizure and epilepsy type, neurological and systemic co-morbidities, MCD), mode of inheritance, germline or somatic mutation, functional characterisation, and year of first published case or case series.

3.2.2 Systematic review of evidence supporting neuronal mTOR pathway hyperactivation as an important pathomechanism in mTOR-related epilepsies

Articles reporting evidence of neuronal mTOR pathway hyperactivation were identified using a PubMed literature search *(date 14th, November 2022)* using the following terms:

- (epilepsy) OR (seizure) *AND*
- (mTOR) OR (mechanistic target of rapamycin) OR (mammalian target of rapamycin) *AND*
- (hyperactivation) OR (hyperactivity) OR (excessive activation)

Total results= 193

Abstracts and references were reviewed to identify studies in which molecular evidence of mTOR pathway hyperactivation was demonstrated in either (a) preclinical models of mTOR-related epilepsies or (b) brain resections from epilepsy patients with mTOR gene mutations.

3.3 Results

3.3.1 Epilepsy-associated mTOR pathway disease genes: discovery over time, phenotypes and genotypes

This review of literature identified 17 epilepsy-associated disease genes encoding mTOR pathway proteins (see **Figure 3.2**). In 1993, the *TSC2* gene was the first mTOR gene to be cloned. This was facilitated by characterisation of large overlapping deletions involving the *TSC2* locus (previously mapped to chromosome 16p13) from five patients with clinically diagnosed TSC[220]. Between 2012 and 2016, 11 mTOR pathway genes were identified as causes of epilepsies and MCD. This period of accelerated mTOR gene discovery coincided with increased availability of NGS approaches and greater understanding on the role of somatic mosaicism in the development of neurological disease[221]. The most recently described epilepsy-associated mTOR gene is *PIK3C2B,* encoding PI3K-C2β in the growth factor arm of the mTOR cascade. Genetic variants in *PIK3C2B* cause non-lesional focal epilepsies[222]. A comprehensive review of the genetic and phenotypic features of mTOR-related epilepsies is found in **Table 3.1**.

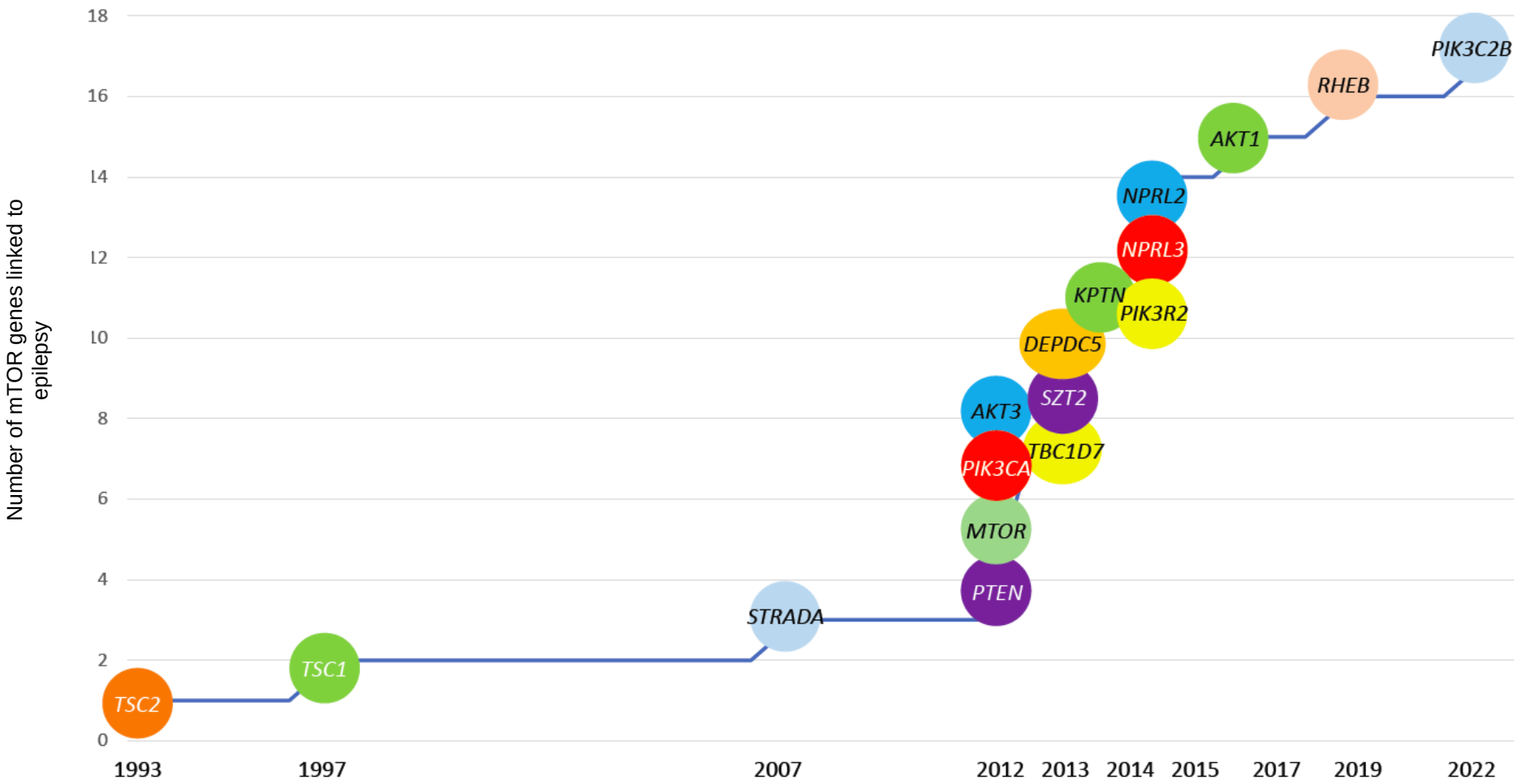

Figure 3.2 mTOR pathway gene discovery timeline

Footnotes:
Germline *PTEN* mutations were first recognised as a cause of autism in 2001[223], while somatic *AKT1* variants were identified as the cause of Proteus syndrome in 2011[224].

The mTORopathies comprise of a spectrum of MCD that range from whole brain (megalencephaly) and hemispheric (HME) abnormalities to focal abnormalities such as FCD and bottom of-sulcus dysplasia (BOSD), to 'normal' appearing brain on high resolution MRI. Pathogenic variants in GATOR1 genes most commonly cause non-lesional focal epilepsies[127]. Epilepsy may result from subtle FCD that is not detectable by conventional imaging techniques in an unknown proportion of people with GATOR1 mutations and normal high-resolution MRI[225]. mTOR-related MCD is often associated with ID and/or ASD.

Some mTORopathies are multisystem disorders (for example, TSC), whilst others have a 'brain only' phenotype (for example, *DEPDC5*-related epilepsies) (see **Table 3.2**). The mechanisms that dictate the pattern of organ involvement in the various mTORopathies are poorly understood, but may relate to organ-specific gene expression, the normal function of the faulty protein within the mTOR cascade, and the timing of mutagenesis. In addition to the more prevalent mTORopathies, like TSC and the GATOR1-related epilepsies, there are several very rare multisystem disorders including polyhydramnios, megalencephaly and symptomatic epilepsy (PMSE), Smith–Kingsmore syndrome, megalencephaly polydactyly polymicrogyria-hydrocephalus syndrome (MPPH) and megalencephaly capillary malformation-polymicrogyria syndrome (MCAP)[215, 226-228].

The mTORopathies share common neuropathological features including abnormal cellular morphology and enlargement (cytomegaly), disorganised cortical lamination, neuronal hyperexcitability and constitutive mTORC1 signalling[229]. FCD type II is a major cause of childhood-onset DRE and is categorised as an mTORopathy based on its molecular and cellular traits. FCD type IIA is characterised by cortical dyslamination and dysmorphic neurons. FCD type IIB is distinguished from FCD type IIA by the presence of balloon cells. HME is at the severe end of the FCD spectrum and is characterised by enlargement of part or all of one hemisphere, often with histological features of FCD type II[230]. BOSD is at the milder end of the spectrum, with signal change and cortical thickening at the bottom of a sulcus on MRI and histological features consistent with FCD type II[158]. Cortical and subcortical tubers in TSC have been reclassified as FCD, as they share histopathological features with FCD type II, including disorganised lamination and dysmorphic cytomegalic neurons[230].

Low-level mosaic somatic mutations in mTOR pathway activating genes (*MTOR, AKT3, PIK3CA, RHEB*) are a major cause of FCD type II and HME[90, 91, 142, 154, 158, 221, 231, 232]. Germline *TSC1*, *TSC2, PTEN, DEPDC5, NPRL2* and *NPRL3* variants are also associated with FCD type II and HME[90, 127, 142, 232]. Knudson's two-hit mechanism is proposed for FCD seen in patients with germline mutations in mTOR pathway genes[233]. Second-hit somatic mutations have been demonstrated in surgically resected FCD (including HME and tubers) from patients with germline *TSC1, TSC2* and *DEPDC5* variants[90, 91, 142-144, 234-236]. Germline homozygous *DEPDC5* variants have recently been reported in patients with severe early onset refractory epilepsy, ID, macrocephaly and bilateral perisylvian polymicrogyria[237].

Table 3.1: Epilepsy-associated mTOR pathway genes: genotypes and phenotypes

mTOR protein	Normal effect on mTORC1	Disease gene and inheritance	Epilepsy Syndrome	Malformation of cortical development
TSC1	Inhibition (*part of TSC protein complex*)	Germline *TSC1* (Dominant)	TSC; Focal epilepsy with structural cause *(see next column)*	Cortical tuber FCD type II HME[142, 156]
		Somatic *TSC1*	TSC; Focal epilepsy with structural cause (*see next column)*	Cortical tuber FCD type II
TSC2	Inhibition (*part of TSC protein complex*)	Germline *TSC2* (Dominant)	TSC; Focal epilepsy with structural cause *(see next column)*	Cortical tuber FCD type II HME[142, 156]
		Somatic *TSC2*	TSC; Focal epilepsy with structural cause *(see next column)*	Cortical tuber FCD type II *Second hit somatic mutations in tubers and HME*[142]
TBC1D7	Inhibition (*part of TSC protein complex*)	Germline *TBC1D7* (Recessive)	No seizures reported but epileptiform discharges on EEG[238]	Megalencephaly[239]
DEPDC5	Inhibition (*component of GATOR1 complex*)	Germline *DEPDC5* (Dominant)	Focal epilepsy with no structural cause detectable on MRI brain; Focal epilepsy with structural cause *(see next column)*	FCD type II[90] BOSD[158] HME[142] FCD type I[127] Subcortical band heterotopia[240] Polymicrogyria[130] Pachygyria[241]
		Germline *DEPDC5* (Recessive)	Infantile-onset DEE	Bilateral perisylvian polymicrogyria[237]
		Somatic *DEPDC5*	Focal epilepsy with structural cause *(see next column)*	*Second hit mutations in FCD type I and type II*[90, 142, 143, 234]
NPRL2	Inhibition (*component of GATOR1 complex*)	Germline *NPRL2* (Dominant)	Focal epilepsy with no structural cause detectable on MRI brain; Focal epilepsy with structural cause *(see next column)*	FCD type II[127] FCD type I[131] Polymicrogyria[130]
NPRL3	Inhibition (*component of GATOR1 complex*)	Germline *NPRL3* (Dominant)	Focal epilepsy with no structural cause detectable on MRI brain[130]; Focal epilepsy with structural cause *(see next column)*	FCD type II[131] BOSD[158] HME[242] FCD type I[127]

mTOR	Activation (combines with binding partners to form mTORC1)	Germline *MTOR* (Dominant)	Focal epilepsy with no structural cause detectable on MRI brain[243]; Smith-Kingsmore syndrome *(seizures in 73.9% of cases)*[226]	HME Megalencephaly[226]
		Somatic MTOR	Focal epilepsy with structural cause *(see next column);* Smith-Kingsmore syndrome[226]	FCD type II[90, 91] BOSD[158] HME[90, 91] Megalencephaly[226] Polymicrogyria[244]
Akt	Activation (*component PI3K-Akt pathway*)	Germline *AKT3* (Dominant)	MPPH syndrome *(seizures in 47% of cases)*[245]	Polymicrogyria Megalencephaly[227, 228]
		Somatic *AKT1*	Focal epilepsy with structural cause *(see next column);* Proteus syndrome *(seizures rarely reported)*[224]	HME[142] Megalencephaly[224]
		Somatic *AKT3*	Focal epilepsy with structural cause *(see next column)*	FCD type II[90, 91] HME[90, 142, 154, 240]
PI3K	Activation (*component PI3K-Akt pathway*)	Germline *PIK3CA* (Dominant)	MCAP syndrome *(seizures in 20% of cases)*[245]	Megalencephaly Polymicrogyria[246]
		Germline *PIK3R2* (Dominant)	MPPH syndrome *(seizures in 47% of cases)*[227, 245]; Focal epilepsy with structural cause *(see next column)*	Megalencephaly Polymicrogyria[167]
		Germline *PIK3C2B* (Dominant)	Focal epilepsy with no structural cause detectable on MRI brain[222]	None
PI3K *(cont.)*	Activation (*component PI3K-Akt pathway*)	Somatic *PIK3CA*	Focal epilepsy with structural cause *(see next column)*; MCAP syndrome *(seizures in 20%)*[245]; MPPH syndrome (*seizures in 47%*)[227, 245]	FCD type II[154] HME[90, 91] Megalencephaly[154] Polymicrogyria[245]
		Somatic *PIK3R2*	Focal epilepsy with structural cause *(see next column)*	Megalencephaly Polymicrogyria[167]
STRADα	Inhibition	Germline *STRADA* (Recessive)	PMSE syndrome (infantile-onset DEE)	Megalencephaly Subependymal dysplasia[215]

PTEN	Inhibition of PI3K-Akt pathway	Germline *PTEN* (Dominant)	Focal epilepsy with structural cause *(see next column);* Bannayan-Riley-Ruvalcaba syndrome (*seizures occur in 25% of cases*)[247]; Cowden syndrome *(seizures reported in some cases)*[248]; ASD and macrocephaly syndrome (*seizures reported in some cases*)[249]	FCD[250] HME[154] Megalencephaly[249] Polymicrogyria[249] Subependymal heterotopia[249]
		Somatic *PTEN* (Biallelic)	Focal epilepsy with structural cause *(see next column)*	*Two distinct somatic mutations in a case of HME*[157]
Rheb	Activation	Somatic *RHEB*	Focal epilepsy with structural cause *(see next column)*	FCD type II[90] HME[251]
SZT2	Inhibition *(part of KICSTOR complex)*	Germline *SZT2* (Recessive)	Infantile-onset DEE[252]	Megalencephaly
Kaptin	Inhibition *(part of KICSTOR complex)*	Germline *KPTN* (Recessive)	Infantile-onset DEE[253]	Megalencephaly

Abbreviations:
ASD= autism spectrum disorder; BOSD= bottom-of-sulcus dysplasia; DEE= developmental and epileptic encephalopathy; FCD= focal cortical dysplasia; HME= hemimegalencephaly; MCAP= megalencephaly-capillary malformation-polymicrogyria; MPPH= megalencephaly-polydactyly-polymicrogyria-hydrocephalus; PMSE= polyhydramnios, hydrocephalus and symptomatic epilepsy.

Table 3.2: 'Brain only' versus multisystem mTORopathies

'Brain only' mTORopathies

- GATOR1-related focal epilepsies (germline *DEPDC5, NPRL2* and *NPRL3)*
- Focal cortical dysplasia type II (somatic *MTOR, AKT3, PIK3CA and RHEB)*
- Hemimegalencephaly (somatic *MTOR, AKT3, PIK3CA, RHEB* and *PTEN)*
- Infantile-onset developmental and epileptic encephalopathies (germline *SZT2* and *KPTN)*[a]
- *PIK3C2B*-related non-lesional focal epilepsies

Multisystem mTORopathies

- Tuberous sclerosis complex (germline and somatic *TSC1* and *TSC2)*
- Smith-Kingsmore syndrome (germline and somatic *MTOR)*[a]
  - Dysmorphic facial features, intellectual disability, herniae, hypomelanosis
- Polyhydramnios, megalencephaly, and symptomatic epilepsy (germline *STRADA)*[a]
  - Polyhydramnios, facial dysmorphism, intellectual disability, skeletal deformity, cardiac anomalies
- Megalencephaly-capillary malformation syndrome (germline and somatic *PIK3CA*)[a]
  - Cutaneous vascular malformations, intellectual disability, digital abnormalities, cardiac anomalies
- Megalencephaly-Polymicrogyria-Polydactyly-Hydrocephalus (germline *AKT3* and *PIK3R2*)[a]
  - Intellectual disability, postaxial polydactyly, facial dysmorphism
- Proteus syndrome (somatic *AKT1*)[a]
  - Patchy or segmental overgrowth and hyperplasia of multiple tissues
- Bannayan-Riley-Ruvalcaba syndrome (germline *PTEN*)[a]
  - Childhood onset, macrocephaly, lipomas, hamartomas, intellectual disability
- Cowden syndrome (germline *PTEN)*[a]
  - Adult onset, multiple hamartomas, increased cancer risk, Lhermitte-Duclos disease, macrocephaly
- TBC1D7-related macrocephaly (germline *TBC1D7)*[a]
  - Patellar dislocation, osteoarticular anomalies, coeliac disease, intellectual disability

Footnotes:
[a] Very rare mTORopathies

3.2.2 Evidence supporting neuronal mTOR pathway hyperactivation as an important pathomechanism in mTOR-related epilepsies

The precise mechanisms by which mTORopathies cause neuronal hyperexcitability and seizures remain to be fully defined, but excessive mTORC1 activation appears to be implicated in epileptogenesis by disrupting the formation of neural circuits and by altering established neural networks[225]. The extent to which seizures are a direct consequence of mTORC1 hyperactivation rather than a corollary of network disruption due to structural cortical malformation remains unresolved. In a rodent model of biallelic *Tsc1* deletion, mice developed early severe seizures, without significant alteration of brain structure, supporting the theory that excessive mTOR activation alone is sufficient to generate seizures[254].

mTOR pathway activity is assessed by immunostaining for downstream substrates of mTORC1 activation, such as phosphorylated ribosomal S6K. Hyperactivity of the mTORC1 pathway has been demonstrated in:

a) Experimental animal models of mTORopathies.
b) *In vitro* functional assessments of epilepsy-causative mTOR pathway genetic variants.
c) Resected brain tissue from patients with FCD and HME.

3.2.3 Animal Models of mTORopathies

Preclinical rodent models of mTORopathies have been developed to study the effects of knockdown or conditional knockout of mTOR pathway inhibitors or overexpression of mTOR pathway activators. Gene knockout involves the permanent alteration of DNA through experimental manipulation in a laboratory setting, resulting in LoF of the gene. Conditional knockout is a technique that eliminates gene expression in a specific organ, tissue, or cell. Gene knockdown is a technique used in experimental settings to decrease the expression of one or more genes in an organism.

Conditional knockout models of *Pten, Tsc1* and *Tsc2* were associated with disorganised cortical cytoarchitecture, cytomegalic neurons, mTORC1 hyperactivation and rapamycin-responsive seizures[255-257] In TSC models, the neurological phenotype was almost completely prevented by early treatment with rapamycin[256, 257].

Heterozygous rodent models of TSC often lack the neuropathological features and spontaneous seizures of human disease, supporting that second-hit somatic mutations may be necessary for clinical disease. *Strada* knockdown in a mouse model of PMSE resulted in ventricular heterotopic neurons. Rapamycin rescued the cortical migratory defect[215]. Murine FCD models have been developed using *in utero* electroporation to produce focal cortical expression of mutant *Mtor* and *Rheb*. Mutant mice with FCD displayed dysmorphic neurons and spontaneous seizures, both almost completely rescued by rapamycin treatment[231, 258].

Both global and conditional knockout of *Depdc5* in rodent models led to cytomegalic dysmorphic neurons, hyperexcitable cortical neurons, markers of mTORC1 upregulation and lowered seizure thresholds[259, 260]. Experimental models of FCD developed using *in utero* electroporation combined with CRISPR-Cas9 editing of *Depdc5* in rodent cortex resulted in enlarged neurons, hyperactivated mTORC1, clinical seizures and sudden death[143, 261]. In another *Depdc5* conditional knockout model, mice displayed increased phosphorylated S6K immunostaining, thickened cortex, cytomegalic neurons, clinical seizures and premature death. Rapamycin reduced seizure frequency and extended survival in this *Depdc5* model[262]. *Nprl2*- and *Nprl3*-knockout mice displayed similar features to *Depdc5* rodent models: seizures, dysmorphic enlarged neuronal cells and increased mTORC1 activity. Rapamycin reduced seizures and lengthened survival, although the treatment benefit was less durable compared with *Depdc5*-knockout mice[263].

Zebrafish models of TSC and *DEPDC5*-related epilepsy have also been developed. Biallelic *Tsc2* models of TSC displayed increased mTORC1 activity, structural brain lesions and spontaneous epileptiform events, whereas monoallelic models lack these TSC-like features[264, 265]. A zebrafish model of *Depdc5* knockout showed spontaneous epileptiform events, mTORC1 hyperactivity and premature death[266]. *Depdc5* knockdown zebrafish displayed early onset motor and neuronal hyperactivity, consistent with seizures. Rapamycin treatment reduced the seizure-like episodes[267]. A double mutant zebrafish model carrying LoF mutations in both *Tsc2* and *Depdc5* displayed increased mTORC1 hyperactivity and greater susceptibility to seizures compared with single mutant models, suggesting that a second-hit LoF mutation in a

different mTOR gene could contribute to disease in TSC or *DEPDC5*-related epilepsy[268].

3.2.4 *In vitro* functional assessment of disease-causing mTOR gene variants

In vitro functional assessments of mTOR pathway genetic variants detected in patients with epilepsy demonstrated molecular evidence of mTORC1 hyperactivation. mTORC1 functional assays can be performed on transfected heterologous systems (for example, human embryonic kidney cells) or iPSCs. Functional assessments of some *TSC1*, *TSC2, DEPDC5, NPRL2, NPRL3, SZT2* and *STRADA* variants (all genes encoding negative regulators of mTORC1) were associated with increased mTORC1 activity[215, 269-272]. *In vitro* studies of genes encoding pathway activators, such as *AKT3*, *PIK3R2, PIK3CA* and *MTOR* also displayed increased immunostaining for downstream substrates of mTORC1[227, 231]. Three-dimensional cerebral organoid models of TSC and PMSE generated from patient-derived iPSCs better recapitulate human disease and show evidence of mTORC1 hyperactivity[273, 274]. Importantly, a significant proportion of the GATOR1 variants studied *in vitro* (just under 70%) were not associated with increased phosphorylated S6K immunostaining, which brings into question the pathogenicity of some GATOR1 missense variants[270, 271]. However, these functional assays may not reflect the *in vivo* behaviour of some GATOR1 variants and other aspects unrelated to mTORC1 signalling may produce the epilepsy phenotype.

3.2.5 Resected human brain tissue

Resected FCD type II and HME specimens consistently demonstrate evidence of enhanced constitutive mTORC1 activation[144, 275-277] Increased mTORC1 activity has been shown in FCD type II and HME specimens from patients with somatic mutations in mTOR pathway activating genes (*MTOR, PIK3CA, AKT3* and *RHEB*)[90, 153, 154, 158, 231]. Resected FCD type II and HME specimens from patients with germline GATOR1 variants (*DEPDC5, NPRL2* and *NPRL3*) have also displayed enhanced phosphorylated S6K expression[90, 131, 134, 158, 278]. Importantly, FCD type II specimens display evidence of mTORC1 hyperactivation, irrespective of the presence of detectable somatic or germline variants, consistent with the hypothesis that all FCD type II are mosaic mTORopathies[90].

3.4 Discussion

In this chapter I have shown that mTORopathies comprise a wide spectrum of epilepsies ranging from non-lesional focal epilepsy to epilepsy caused by FCD or HME, to DEE with 'brain only' or multisystem manifestations. The mTOR-related epilepsies are caused by pathogenic variants in a diverse range of mTOR pathway genes (17 and counting), with different inheritance patterns (dominant, recessive, *de novo*), and distinctive modes of mutagenesis (germline, somatic or double hit). I also demonstrated that neuronal mTORC1 hyperactivation is a shared molecular feature of all mTORopathies, as demonstrated in: a) preclinical animal models; b) *in vitro* functional assessments of heterologous cell lines, iPSCs and cerebral organoid models; and c) resected brain tissue from patients with mTORopathies. Significantly, rapamycin treatment improved seizures and other neurodevelopmental phenotypes in several preclinical models of mTORopathy, including those of GATORopathy. This finding serves as the most compelling proof that mTORC1 hyperactivation is responsible for causing seizures and MCD in mTORopathies.

The finding that mTOR inhibitor treatment can reverse epilepsy and other neurodevelopmental traits in rodent and zebrafish models of GATORopathy and FCD type II has important therapeutic implications[231, 258, 262, 263, 267]. More efficacious therapies for GATOR1-related epilepsies are needed, as these disorders are frequently associated with DRE and a disproportionate risk of SUDEP[127]. As mTOR inhibitors have proven to be efficacious and safe treatments for DRE in TSC, they represent a promising therapeutic strategy in GATOR1-related epilepsies.

All mTOR pathway genes (and encoded proteins) described above are part of the "canonical" mTOR pathway. Recent findings indicate that certain conditions induce selective mTORC1 activity directed at specific substrates. The so-called "non-canonical" mTOR pathway modulates selective mTORC1 activity in response to stimuli that converge on the lysosomal surface. Dysregulated non-canonical mTORC1 signalling underlies the pathogenesis of rare neurodevelopmental disorders and epilepsies[279]. Transcription factor E3 (TFE3) plays an important role in lysosomal biogenesis and autophagy. TFE3 activity is modulated by caloric depletion, DNA

damage, mitochondrial damage and pathogens, via mTORC1-dependent mechanisms. X-linked *de novo* variants in *TFE3* cause a distinctive syndrome comprising ID, pigmentary mosaicism (pigmentation anomalies along Blaschko's lines), and occasionally epilepsy[280].

Rab GTPase-activating protein 1 (RABGAP1) is another non-canonical mTOR regulator involved in intracellular lysosomal positioning and vesicular trafficking to promote mTORC1 signalling. Biallelic variants in *RABGAP1* cause a novel neurodevelopmental phenotype including ID, microcephaly, sensorineural hearing loss, seizures, thinning of the corpus callosum and dysmorphic facial features[281]. This is the first mTOR-related neurodevelopmental disorder to demonstrate downregulated mTOR signalling as a potential mechanism underlying the disease.

The precise mechanisms by which excessive mTORC1 activation leads to the development of epileptiform discharges and seizures have yet to be fully elucidated. Dysmorphic neurons and balloon cells are considered a neuropathological hallmark of aberrant mTOR signalling, as they consistently display enhanced mTORC1 activation and are the main carriers of somatic mutations in FCD[90, 275, 276]. *In vitro* electrophysiological studies of FCD tissue have shown that dysmorphic and immature neurons play an important role in the generation and propagation of epileptic discharges, while balloon cells lack epileptogenicity[282]. Moreover, FCD type II and cortical tubers retain immature GABA signalling mechanisms, resulting in abnormal neural networks and hyperexcitable cortical foci[283, 284]. In the immature CNS, GABA acts as an excitatory neurotransmitter, in contrast to its inhibitory function in the developed CNS[285]. Retention of immature GABA receptors leads to the development of spontaneous pacemaker GABA receptor-mediated synaptic activity, which results in the generation of self-sustaining abnormal epileptogenic discharges[286].

Deficits in mTOR-regulated autophagy may also contribute to abnormal GABAergic signalling. Under normal physiological conditions, GABA receptor-associated proteins (GABARAP) play an important role in facilitating inhibitory signals between GABAergic interneurons and neurons. However, in autophagy-deficient neurons, sequestration of GABARAP reduces the protein's ability to mediate GABA receptor trafficking, resulting in hyperactivated neurons[287]. In the brains of patients with TSC, many GABA type A

receptor subunits are downregulated, which probably provides a neuroanatomical substrate for the early appearance of seizures and encephalopathy[257].

In a zebrafish model of *Depdc5* knockout displaying spontaneous epileptiform events and premature death, fine branching of the GABAergic network was particularly affected, suggesting a potential contribution to epileptogenesis[266]. A transcriptomic analysis revealed specific downregulation of GABA receptors, transporters, and factors associated with inhibitory network development, synapse formation, and function, suggesting that DEPDC5 may play a role in the formation, pruning, and maintenance of the GABAergic system. Treatment with vigabatrin, a drug that increases endogenous GABA levels by inhibiting its metabolism, successfully rescued the phenotype. Interestingly, a comparison of transcriptomic datasets showed a significant overlap between *Depdc5* and *Gabra1* datasets, independent of the *Tsc2* dataset. These findings suggest that DEPDC5 may play an important role in controlling neurodevelopmental aspects of GABAergic networks in an mTOR-independent manner[266].

Abnormalities in dendritic spine morphology and glutamatergic synaptic transmission were observed in rodent models of *Tsc1* and *Tsc2* knockout. These alterations in neuronal structure and function likely contribute to the pathogenesis of epilepsy in TSC[288]. In a mouse model of *DEPDC5*-related FCD involving double-hit variants, *Depdc5* inactivation resulted in abnormal dendritic and spine shaping, increased excitatory transmission and epileptogenesis[143].

Having shown that excessive mTOR activation plays an important role in epileptogenesis in all mTORopathies, the next chapter will focus on the clinical pharmacology of available mTOR inhibitors, and a scoping review of mTOR inhibitor treatment for neurological manifestations of mTORopathies.

4. mTOR inhibitor treatment for epilepsies in mTORopathies

4.1 Introduction

In 1964, a group of Canadian scientists travelled to the Chilean territory of Easter Island (known as Rapa Nui by its inhabitants) to study its population and biosphere. During the expedition, it was observed that the Easter Island natives had a low incidence of tetanus, despite walking barefoot. The Canadian scientists hypothesised that the soil on Easter Island contained unique antimicrobial compounds. The Hungarian microbiologist Georges Nógrády brought soil samples back to Montréal for analysis. At the Ayerst Laboratory in Montréal, an Indian scientist named Suren Neth Sehgal led research efforts to identify novel antimicrobial properties contained in this natural source[289].

In 1972, Sehgal and his team isolated a strain of *Streptomyces hygroscopicus* from the soil samples collected on Easter Island. This strain was found to produce a molecule with antifungal properties. The compound was named rapamycin after its place of origin, Rapa Nui. Subsequent studies revealed rapamycin's immunosuppressive activity (inhibition of antigen-induced T-cell and B-cell proliferation), which prevented further development as an antifungal agent[290]. Later, Sehgal's team uncovered rapamycin's antiproliferative properties. Samples were sent to the National Cancer Institute for anti-tumour activity screening. Rapamycin was found to inhibit the growth of several tumour cell lines, and gained status as a priority drug. However, the Ayerst Montréal laboratory was closed in 1982, pausing Sehgal's research efforts for some time. Despite this, Sehgal persevered by smuggling samples of *Streptomyces hygroscopicus* in ice-cream containers to his home, where they were stored in his freezer for several years[289, 290].

In 1987, American Home Products merged its Wyeth and Ayerst divisions, allowing Sehgal to push rapamycin back onto their research agenda. Rapamycin's immunosuppressive activity was repeatedly demonstrated through animal testing, and successful clinical studies followed. In 1999, rapamycin (also known as sirolimus) obtained FDA approval for prophylaxis of kidney transplant rejection[289]. Studies to

further elucidate rapamycin's anti-tumour activity confirmed its inhibitory effect on cell growth in a variety of organisms ranging from the environmental yeast *Saccharomyces cerevisiae*, to the fruit fly species *Drosophila,* to a number of mammalian species, including human cells. These inhibitory mechanisms were found to be highly conserved across species, converging on a group of target proteins, collectively termed target of rapamycin (TOR), or mTOR in mammalian cells[291]. In 2002, the function of TSC2 protein (Tuberin) as an inhibitor of mTOR signalling was established, confirming the role of dysregulated mTOR activity in the pathogenesis of TSC[292]. The rapamycin origin story illustrates the importance of serendipity in scientific discovery, and the contribution of a scientist with unwavering persistence and belief in the potential of a novel molecule. Sehgal received lifetime achievement awards from the Indian Society of Organ Transplantation and the Canadian Transplantation Society. He was diagnosed with metastatic colon cancer in 1998, and sirolimus treatment for liver metastasis likely extended his life. He died in 2003, following 40 years of active research uncovering the immunosuppressive and antiproliferative properties of rapamycin[293]. The final frontier in rapamycin research may lie in unlocking its potential as an anti-aging agent. Recent studies in mice have indicated that rapamycin has the potential to extend lifespan by 10%, whilst also preserving frailty scores and speed of gait[294].

Rapamycin and its analogues (or so called 'rapalogues') are now established immunosuppressive agents, utilised in solid organ transplantation for their anti-rejection properties. Their use also extends to the treatment of many cancers including renal cell carcinoma, breast cancer and pancreatic neuroendocrine tumours. The newly established therapeutic benefits of mTOR inhibitors for TSC represent a recent development in this field. mTOR inhibitors are efficacious treatments for several TSC-associated lesions including sirolimus for pulmonary lymphangioleiomyomatosis (LAM) and renal angiomyolipoma (AML)[295], everolimus for SEGA not amenable to surgery[296], and everolimus for AML[297]. The EXIST-3 trial was the first large scale precision medicine trial for genetically mediated epilepsy. In this randomised, double-blind, placebo-controlled study, treatment with everolimus significantly reduced seizure frequency in individuals with TSC-related DRE[135].

The aims of this chapter are:

a) To review the clinical pharmacology and side-effect profile of available mTOR inhibitors.
b) To evaluate clinical evidence supporting the safety and efficacy of mTOR inhibitors for treatment of seizures and other neurological manifestations in mTORopathies.
c) To assess the safety of using mTOR inhibitors during the Coronavirus disease 2019 (COVID-19) pandemic.

4.2 Methods

4.2.1 The clinical pharmacology and side-effect profile of available mTOR inhibitors

Articles reporting data on the clinical pharmacology and side-effect profile of mTOR inhibitors were identified using a PubMed literature search *(date 11th, March 2021)* using the following terms:

- (mTOR inhibitor) OR (everolimus) OR (rapamycin) *AND*
- (Clinical pharmacology) OR (pharmacokinetics) *AND*
- (Adverse events)

Total results= 448

A review of abstracts and references was conducted to identify research studies and reviews, that report on the clinical pharmacology and treatment-emergent adverse events of available mTOR inhibitors. The results of this systematic analysis are reported in Moloney *et al* (2021)[136].

4.2.2 Systematic review of clinical evidence on the safety and efficacy of mTOR inhibitors for treatment of seizures and other neurological manifestations in mTORopathies

Clinical studies evaluating the efficacy and safety of everolimus or sirolimus for seizures and other neurological symptoms in different mTORopathies were identified using a PubMed literature search *(date 12th, December 2022)* using the following terms:

- (mTOR inhibitor) OR (sirolimus) OR (everolimus) *AND*
- (Tuberous sclerosis) OR (focal cortical dysplasia) OR (mTORopathy) *AND*
- (Epilepsy) OR (seizure) OR (neurology)

Total results= 475

Abstracts and references were reviewed to identify relevant clinical studies. The review was narrowed to 35 research papers that reported seizure outcomes or other neurological outcomes in patients with mTOR-related genetic disorders treated with everolimus or sirolimus. I summarised the methods and outcomes of clinical studies with the highest level of research evidence relevant to each mTORopathy.

4.2.3 Review on the safety of using mTOR inhibitors for epilepsy in mTORopathies during the COVID-19 pandemic

Clinical studies evaluating the safety of everolimus or sirolimus use during the COVID-19 pandemic were identified using a PubMed literature search *(date 7th, June 2021)* using the following terms:

- (Sirolimus) OR (everolimus) *AND*
- (COVID-19) OR (SARS-CoV-2) OR (coronavirus)

Total results= 61

Abstracts were reviewed to identify research studies or review articles that evaluated the safety of mTOR inhibitor use during the COVID-19 pandemic. The findings from this review are summarised in Moloney and Delanty, 2021[298].

4.3 Results

4.3.1 The clinical pharmacology and side-effect profile of available mTOR inhibitors

Rapamycin (sirolimus) and rapalogues (everolimus, temsirolimus, ridaforolimus) allosterically inhibit mTORC1 activity by forming a binding complex with FKBP12. Rapalogues were developed to enhance the pharmacokinetic profile of their parent compound sirolimus. Rapalogues all share a central macrolide chemical structure, with different functional groups added at position 40 of the rapamycin structure (see **Figure 4.1**)[299]. Everolimus is a hydroexyethyl ester derivative of sirolimus, while ridaforolimus is a dimethylphosphinate derivative. Both are biochemically active without modification. In contrast, temsirolimus is a prodrug of sirolimus, that requires removal of the dihydroxymethyl propionic acid ester group to become biochemically active[299].

These structural differences affect the bioavailability and half-life of different rapalogues (see **Table 4.1**). Everolimus has a more favourable pharmacokinetic profile compared to sirolimus due to its increased oral bioavailability and slower hepatic metabolism[300]. Temsirolimus is a once weekly intravenous formulation approved for treatment of advanced renal cell carcinoma[301]. Ridaforolimus (also known as deforolimus) is an investigational mTOR inhibitor, with some evidence supporting its use for treatment of metastatic sarcoma[302]. Available mTOR inhibitors display limited penetration across intact blood-brain-barrier, which may limit their efficacy in neurological disorders[299].

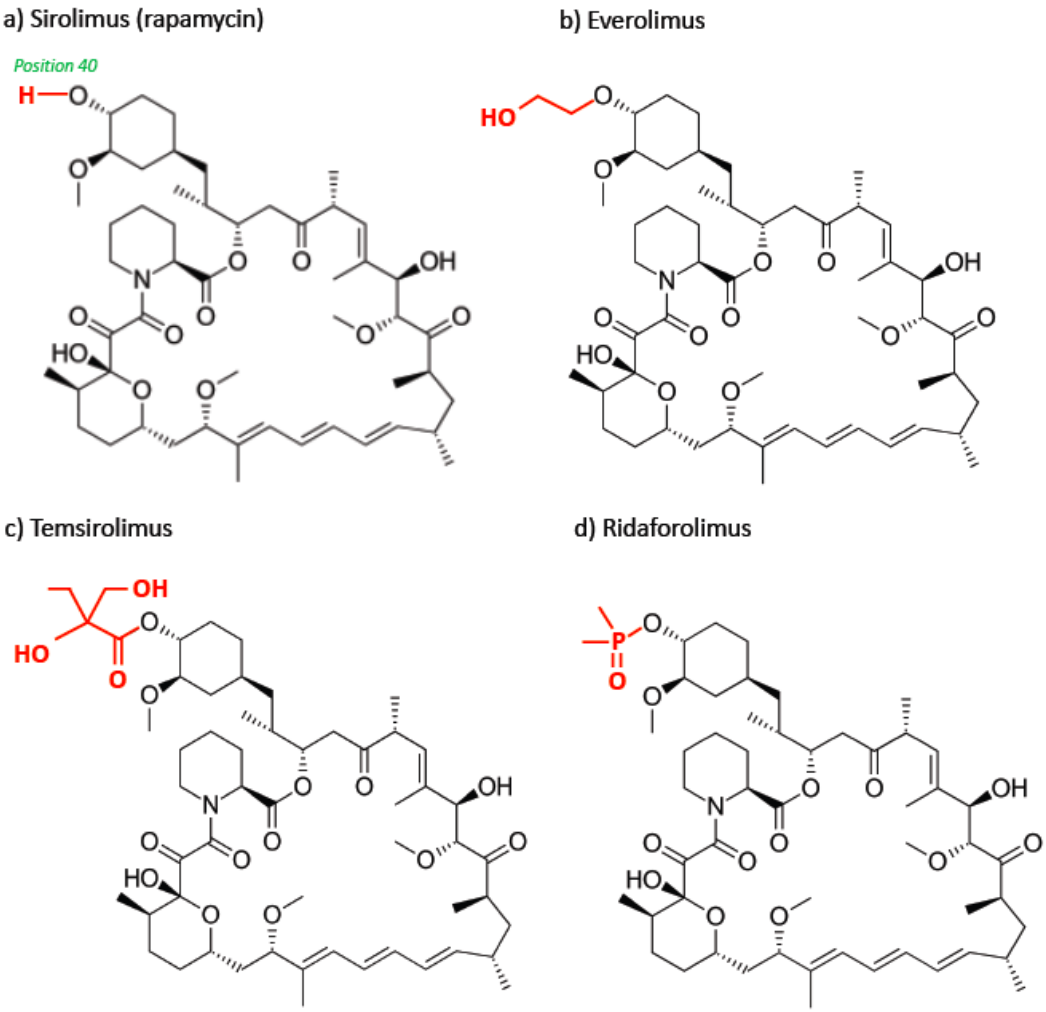

Figure 4.1 The chemical structure of sirolimus and its analogues.

Sirolimus, everolimus, temsirolimus and ridaforolimus all share a central macrolide structure, but each has a unique functional group attached at position 40.

Table 4.1 Clinical pharmacology of rapamycin and its analogues (or rapalogues)

	Rapamycin (sirolimus)	Everolimus	Temsirolimus	Ridaforolimus (deforolimus)
Biochemically active form[299]	Sirolimus is active form	Active derivative (*hydroxyethyl ester)* of sirolimus	Prodrug of sirolimus (*activated after removal of dihydroxymethyl propionic acid ester*)	Active derivative (*dimethyl-phosphinate*) of sirolimus
Mode of administration	Oral, once daily Topical	Oral, once daily	Intravenous, once weekly	Oral or intravenous
Protein binding[299]	~92%	~75%	~85%	~94%
Bioavailability[299]	~15%	20%	100%	Tablet: 16%
Metabolism	CYP3A4, P-glycoprotein	CYP3A4, CYP3A5, CYP2C8 (*Hepatic metabolism 3-fold lower than sirolimus*)[84]	CYP3A4	CYP3A4, P-glycoprotein
Terminal half-life[299]	46-78 hours	26-30 hours	9-27 hours	30-75 hours
Elimination[299]	Faeces (91%), urine (2%)	Faeces (>90%), urine (2%)	Faeces (82%), urine (5%)	Faeces (88%), urine (2%)
CNS penetration	Crosses BBB (*Preclinical data suggests poor CNS penetration*)[303]	Crosses BBB (*Increased CNS penetration compared with sirolimus*)[303]	Crosses BBB (*Decreased CNS penetration compared with sirolimus*)[304]	Crosses BBB (*No data on CNS penetration compared with other agents*)
FKBP12 binding affinity	-	3-fold reduction in binding compared with sirolimus[305]	Similar binding affinity to sirolimus *(prodrug)*	3-fold reduction in binding compared with sirolimus
TSC-related indications	LAM Facial angiofibromas	AML SEGA Drug-resistant epilepsy	Nil	Nil
Other indications	Prevention of organ transplant rejection, polyhydramnios, megalencephaly and symptomatic epilepsy	Prevention of organ transplant rejection, kidney cancer, breast cancer and pancreatic tumours	Advanced kidney cancer	Investigational (sarcoma)

Abbreviations:
AML= angiomyolipoma; BBB= blood-brain-barrier; CYP= cytochrome P450; FKBP12= FK506 binding protein 1 A 12 kDa; LAM= lymphangioleiomyomatosis; mTOR= mechanistic target of rapamycin; SEGA= subependymal giant cell astrocytoma; TSC= tuberous sclerosis complex.

Everolimus and sirolimus require therapeutic drug monitoring due to their narrow therapeutic index, and high inter- and intra-individual pharmacokinetic variability. Potent inducers of cytochrome P450 3A4 (CYP3A4), like carbamazepine and phenytoin, may lower the serum concentration of everolimus[306]. In the TSC trials, everolimus and sirolimus doses were titrated to a target level of 3-15ng/mL[135, 296, 307]. The recommended starting dose of everolimus for treatment of TSC-related epilepsy is based on the patient's age and use of concomitant CYP3A4 inducing medications (see **Table 4.2**)[308].

Table 4.2 Recommended everolimus starting dose for treatment of drug-resistant epilepsy in tuberous sclerosis complex[308]

Age	Without concomitant CYP3A4 or P-glycoprotein inducer	With concomitant CYP3A4 or P-glycoprotein inducer
<6 years	6 mg/m^2	9 mg/m^2
≥6 years	5 mg/m^2	8 mg/m^2

Stomatitis is the most common dose-limiting complication associated with mTOR inhibitor treatment. Stomatitis refers to mucosal inflammation and ulceration involving the mouth, lips and tongue. mTOR inhibitors cause stomatitis by direct toxic effects to oral mucosa. In oncology and transplant medicine settings, up to 70% of patients treated with mTOR inhibitors develop stomatitis[309]. Since TSC patients are rarely treated with additional immunosuppressive and/or chemotherapeutic agents, the incidence of stomatitis in TSC patients treated with everolimus appears to be lower. In the post-extension analysis of the EXIST-3 cohort, 36% of TSC patients developed stomatitis (median duration of everolimus exposure was 30.4 months), and less than 3% developed severe stomatitis[310]. Stomatitis prevention strategies include using a soft toothbrush and mild toothpaste, salt and baking soda mouth rinses, and avoiding spicy, acidic, hot and hard foods. Severe stomatitis can be treated with topical corticosteroids and anaesthetic preparations. Persistent or recurrent severe stomatitis can be managed with systemic steroids, interruption of mTOR inhibitor treatment or mTOR inhibitor dose reduction[311].

Other common adverse events include hypercholesterolemia (~25%), vomiting and diarrhoea (~20%), acneiform rash (~15%), upper respiratory tract infection and nasopharyngitis (~15%), hyperglycaemia (~10%), cytopenia (<10%) and non-infectious pneumonitis (<1%)[310, 311]. Infections and haematological complications appear to be more common in transplant and cancer patients compared to TSC patients[299]. Moreover, treatment with sirolimus seems to cause more frequent side-effects compared to everolimus[311].

Regular blood testing is required to monitor for metabolic complications like hypercholesterolemia, hypertriglyceridemia, hyperglycaemia and diabetes mellitus. The pathophysiology of mTOR inhibitor-induced dyslipidaemia is not yet fully understood, but appears to be related to reduced peripheral clearance of lipids[312]. Specifically, mTOR inhibition has been linked to reduced lipoprotein lipase activity, reduced lipid accumulation in adipose tissue and increased lipophagy, a specialised form of autophagy that releases stored lipids into the bloodstream[313]. Potential mechanisms underlying mTOR inhibitor-induced hyperglycaemia include altered pancreatic insulin secretion and insulin resistance[312]. Significant elevations in lipids can be managed by mTOR inhibitor dose reduction or anticholesterol agents. Pravastatin, fluvastatin and rosuvastatin are the preferred statins for patients taking mTOR inhibitors due to their lower likelihood of interactions. Atorvastatin and simvastatin may interact with mTOR inhibitors via competitive inhibition of CYP3A4, which can lead to statin toxicity. Metformin is recommended as the first-line treatment for mTOR inhibitor-induced diabetes mellitus[312]. Emerging evidence suggests that rapalogues exert beneficial effects on atherosclerosis by reducing macrophage numbers in plaques and enhancing endothelial function. These effects may mitigate some of the adverse metabolic consequences associated with dyslipidaemia and hyperglycemia[313].

Non-infectious pneumonitis is a rare complication of mTOR inhibitor treatment, more commonly seen in oncology settings[314]. The long-term incidence of malignancy in solid organ transplant recipients taking mTOR inhibitors is low compared to those taking other immunosuppressive agents[315].

A pooled analysis of the everolimus TSC trials found that 24% of women developed amenorrhea and 17% reported oligomenorrhea[316]. Evidence suggests that menstrual irregularities resolve after withdrawal of mTOR inhibitor treatment[317]. Data from the extension phases of the EXIST trials do not indicate long-term effects on growth or sexual development[310, 318]. Real-world data from the ***T**uber**O**us* **SC**lerosis registry to increase disease **A**wareness (TOSCA) found that TSC patients treated with everolimus displayed age-appropriate sexual maturation[319].

4.3.2 Systematic review of clinical evidence supporting mTOR inhibitor treatment for seizures and other neurological manifestations in mTORopathies.

Everolimus has class I evidence supporting its efficacy and safety as a treatment for DRE and SEGA in TSC (see **Table 4.3**). In the multicentre, randomised, double-blind, placebo-controlled EXIST-3 study, treatment with everolimus significantly reduced seizure frequency in TSC-related DRE. Forty percent of participants treated with high exposure everolimus (serum everolimus level of 9-15 ng/ml) had a greater than 50% reduction in seizure frequency compared with 15% in the placebo group[135]. Increasing and sustained improvements in seizure control were observed in the EXIST-3 extension phase[310, 320].

Everolimus demonstrated a favourable safety and tolerability profile in the EXIST-3 trial. Stomatitis was the most frequently reported complication, experienced to some degree by approximately 40% of participants. The incidence of adverse events decreased over time and complications rarely led to treatment discontinuation, with infrequent reports of serious infection and neutropenia[135, 310]. Real-world evidence from TOSCA supports the safety and tolerability data from the EXIST-3 trial. Over 60% of patients had an adverse event of any grade, of which stomatitis was the most common. Adverse events were manageable with dose reduction or temporary discontinuation, with a 95% retention rate over five years of observation[319]. Similarly, in a survey-based study on the perspectives of TSC patients treated with everolimus, adverse events were reported by 70% of participants. Overall tolerability was acceptable, with retention rates exceeding 80% after three years[321]. Many participants in EXIST-3 failed to achieve their target serum everolimus level, illustrating the

difficulties of dosing to a target level, particularly in patients with ID for whom blood draws may be a source of distress[135].

Phase 3 clinical trial and extension study (EXIST-1) evidence supports the use of everolimus to reduce SEGA volume in TSC[296, 318, 322]. Everolimus treatment showed benefit in SEGA cases with serial radiological growth, and in patients with new or worsening hydrocephalus[296]. In a post-extension analysis of the EXIST-1 study, 64 out of 111 patients (57.7%) had a greater than 50% reduction in SEGA volume after median everolimus exposure of 47.1 months[318]. Approximately 10% of patients stopped treatment due to adverse events[318]. Protracted interruption of mTOR inhibitor treatment in TSC often leads to tumour regrowth or seizure worsening[295, 318, 323]. Consequently, long-term treatment is recommended for TSC-related tumours and seizures.

Two prospective, placebo-controlled trials of everolimus for TSC-related neurocognitive deficits yielded disappointing findings (see **Table 4.3**)[324, 325]. mTOR inhibitor treatment did not improve IQ scores, behavioural symptoms or neuropsychological deficits in TSC patients with ID and/or ASD. These studies were hampered by small sample sizes and short study periods. Moreover, participants received everolimus aged four to 21 years, which may be too late to reverse early life neurodevelopmental deficits. A study of everolimus treatment for neurocognitive problems in TSC (TRON), involving patients aged 16 to 60 years is currently underway in the U.K.[326]. Results from this placebo-controlled trial are yet to be reported. A recent phase 2, multicentre, placebo-controlled study of everolimus for neurocognitive deficits and behavioural symptoms in patients with *PTEN* mutations found no statistically significant differences between groups (everolimus n=24, placebo n=22). However, several secondary outcome measures showed changes in the direction of improvement including non-verbal IQ, verbal memory, social symptoms and sensory processing[327].

Polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE) syndrome is a very rare autosomal recessive multisystem disorder characterised by infantile-onset DRE, severe cognitive impairment, skeletal deformity and craniofacial dysmorphism. Original descriptions of PMSE came from the American Old Order Mennonite

population. All patients had homozygous truncating deletion of exons 9 to 13 in the *STRADA* gene. In total, 16 patients carrying the homozygous *STRADA* deletion have been reported[215]. In addition, six phenotypically similar cases with novel *STRADA* variants have been described[328-331]. STRADα acts as an mTOR repressor in the ATP-sensing arm of the mTOR pathway. Data from *Strada* knockdown mouse models, *in vitro* functional studies of STRADα depletion, and a post mortem PMSE brain specimen demonstrated rapamycin-sensitive aberrant mTORC1 activity[215, 332]. As Old Order Mennonite patients with PMSE share the same deletion, they represent a homogeneous study population. Five patients with PMSE from the Old Order Mennonite community were treated with sirolimus before the onset of epilepsy (started at a mean age of 4.8 months). All patients had reduced seizures and improved receptive language compared to a cohort of historical controls[215]. This early precision medicine trial provided a precedent for further study of mTOR inhibitor therapy for epilepsy and neurodevelopmental disorders in mTORopathies.

Epilepsy surgery is the standard of care for eligible patients with DRE caused by FCD type II and HME. Post-surgical seizure freedom rates approach 70% for FCD type II[333]. Somatic and germline variants in mTOR pathway genes cause FCD type II and HME, with a causative mutation detected in around 60% of cases[90]. FCD type II surgical resections consistently display evidence of enhanced mTORC1 activation, even in specimens where low-level somatic mutations were not detectable[90]. mTOR inhibitors may offer an alternative therapeutic strategy in patients with persistent seizures after epilepsy surgery for FCD, or for surgically inaccessible FCD. A prospective, open-label study of sirolimus for DRE caused by FCD type II was performed to investigate this hypothesis (see **Table 4.3**)[334]. Thirteen participants had a histopathological diagnosis of FCD type II following epilepsy surgery. The other three patients had presumed FCD type II based on characteristic MRI features. Three patients dropped out during the study period: one patient developed SE during the dose-titration phase and two patients were non-compliant with study protocols. One-third of participants had a greater than 50% reduction in seizure frequency, of whom one patient achieved seizure freedom. Although there was a signal toward improved seizure outcomes, the overall seizure frequency reduction did not meet the predetermined level of statistical significance[334]. Four case reports describing patients treated with mTOR inhibitors for HME yielded mixed findings. Two patients had mosaic somatic variants in *MTOR*[335,

[336], and two had germline variants in *NPRL3*[337, 338]. Two of the four patients (one *MTOR* and one *NPRL3*) had reduced seizures on sirolimus, although the infant with *NPRL3*-related HME stopped treatment after three months because of recurrent infections[336, 337]. The other two patients showed no improvement on mTOR inhibitor treatment[335, 338]. Further studies with larger numbers and longer-term follow-up will determine if seizure outcomes improve over time in FCD type II and HME.

Table 4.3 Summary of evidence supporting mTOR inhibitor therapy for epilepsies and other neurological manifestations in mTORopathies

Tuberous sclerosis complex (TSC)		
Seizures		
Phase 1-2, prospective, single-centre, open-label study of **everolimus** for DRE in patients with TSC aged ≥2 years. Study protocol included a 4-week period to establish baseline seizure frequency, a 4-week everolimus dose titration period and an 8-week maintenance treatment period. Everolimus titrated to target level of 5-15ng/mL.	20 patients treated with everolimus (median age was 8 years, range 2-21 years). 60% of patients had a ≥50% reduction in seizure frequency. No patients discontinued treatment due to side-effects. Stomatitis and upper respiratory tract infections were the most reported AE.	Krueger *et al* (2013)[339]
Phase 3, multicentre, randomised, double-blind, placebo-controlled study of **everolimus** for DRE in TSC patients aged 2-65 years (EXIST-3). Participants randomised to placebo, low exposure everolimus (level 3–7 ng/mL) and high exposure everolimus (9–15 ng/mL). Study protocol included an 8-week period to establish baseline seizure frequency, a 6-week everolimus dose titration period and a 12-week maintenance treatment period.	Placebo group (n=119) Low exposure everolimus (n=117) High exposure everolimus (n=130) Median age was 10.1 years (range 2.2-56.3 years) 28.2% of patients in the low exposure everolimus group had a ≥50% reduction in seizures, and 40% in the high exposure everolimus group had a ≥50% reduction. Moderate to severe AE reported in 18% of patients in the low exposure everolimus group, and 24% in the high exposure everolimus group (most commonly, stomatitis).	French *et al* (2016)[135]
EXIST-3 open-label extension study (up to 2 years). **Everolimus** was titrated to a target level of 3-15 ng/mL.	361 patients received everolimus in the extension phase. The response rate (≥50% reduction in seizures) was 31% at 18 weeks, 46.6% at 1 year, and 57.7% at 2 years. 26.3% of participants stopped everolimus during the extension phase, of whom half stopped due to AE. Moderate to severe AE reported in 40.2%, including 2 deaths.	Franz *et al* (2018)[320]
Randomised-controlled trial of **sirolimus** for DRE in children with TSC. Children randomised to immediate adjunctive treatment with sirolimus, or add-on sirolimus after 6 months. Sirolimus was titrated to a target level of 5-10ng/mL.	12 children received add-on sirolimus immediately, and 11 were treated after 6 months. 75% of patients treated with sirolimus had a ≥50% reduction in seizures, including 3 who achieved seizure freedom. However, a significant therapeutic benefit was not observed in the intention-to-treat analysis. 5 patients stopped sirolimus due to AE. Stomatitis and upper respiratory tract infections were common.	Overwater *et al* (2016)[307]

Prospective, open-label observational study of **everolimus** for West syndrome in infants (<12 months) with TSC. All patients failed to respond to vigabatrin and high-dose adrenocorticotropic hormone treatment. Everolimus was titrated to a target level of 5-15ng/mL.	4 infants were treated with everolimus for drug-resistant West syndrome. The median duration of treatment was 13 months. All had hypsarrhythmia on EEG. 2 patients achieved electroclinical remission. Developmental scores improved in 3 patients. No patients discontinued treatment due to AE.	Samueli *et al* (2018)[340]
Subependymal giant-cell astrocytoma (SEGA)		
Phase 1-2, prospective, single-centre, open-label study of **everolimus** for SEGA in TSC aged ≥3 years. Eligible patients had serial growth of SEGA on at least 2 MRI brains. Core treatment phase was 6-months, after which patients entered open-ended extension phase. Everolimus titrated to target level of 5-15ng/mL.	28 patients enrolled in study. 32% of patients had a ≥50% reduction in SEGA volume based on MRI (median duration of everolimus exposure 21.5 months). No patients discontinued treatment due to AE, although stomatitis and upper respiratory tract infections were common.	Krueger *et al* (2010)[341]
Phase 3, multicentre, randomised, double-blind, placebo-controlled study of **everolimus** for SEGA in patients with TSC aged 0-65 years (EXIST-1). Eligible patients had SEGA ≥1cm in diameter, and either serial growth, a new lesion of ≥1cm, or new or worsening hydrocephalus. 3-month study period. Everolimus titrated to target level of 5-15ng/mL.	78 patients treated with everolimus and 39 received placebo. 35% of patients in the everolimus group had a ≥50% reduction in SEGA volume. No patients discontinued treatment due to AE. 32% experienced mild to moderate stomatitis.	Franz *et al* (2013)[296]
Open-label extension of EXIST-1 study. Patients who received **everolimus** or placebo given option to enter extension phase. Everolimus titrated to target level of 5-15ng/mL.	Of the original 117 randomly assigned, 111 received everolimus in the extension phase (median duration of exposure 29.3 months). 49% of patients had a ≥50% reduction in SEGA volume. 5% stopped treatment because of AE.	Franz *et al* (2014)[322]
Open-label postextension analysis of EXIST-1 study. **Everolimus** titrated to target level of 5-15ng/mL	Of the 111 patients who entered the extension phase, 57.7% had a ≥50% reduction in SEGA volume (median duration of exposure 47.1 months). 11 patients stopped treatment because of AE. Stomatitis was the most common AE (43.2%).	Franz *et al* (2016)[318]

TSC-associated neuropsychiatric disorders		
Prospective, randomised, double-blind, placebo-controlled trial of **everolimus** for neurocognitive and behavioural symptoms in TSC. Comprehensive neurocognitive and behavioural evaluation battery performed at baseline, 3 months, and 6 months. Everolimus dose titrated to target level of 5-15ng/mL.	32 patients randomised to receive everolimus, with 15 patients in the placebo group (aged 6-21 years). No significant difference between the two groups at the end of 6 months.	Krueger *et al* (2017)[324]
Prospective, randomised, double-blind, placebo-controlled trial of **everolimus** for full scale IQ, autism, neuropsychological functioning and behavioural problems in TSC. Everolimus dose titrated to target level of 5-10ng/mL. Treatment period of 12 months.	32 patients with TSC-associated intellectual disability and/or autism were randomised (aged 4-17 years). No treatment benefit observed for full-scale IQ, autism, neuropsychological functioning, behavioural problems and quality of life. All patients reported AE and 2 patients stopped everolimus due to AE.	Overwater *et al* (2019)[325]
Substudy of EXIST-3 in Japan. Prospective, randomised, placebo-controlled study of **everolimus** for ASD symptoms assessed using the Pervasive Developmental Disorders Autism Society Japan Rating Scale (PARS) at baseline and week-18.	19 patients with ASD at baseline (11 treated with everolimus and 8 in placebo group). 4 of the 11 treated with everolimus showed an improvement of ≥5-point PARS score. 1 of the 8 in the placebo arm showed an improvement of ≥5-point PARS score.	Mizuguchi *et al* (2019)[342]
Focal cortical dysplasia type II and hemimegalencephaly		
Prospective, open-label study of **sirolimus** for DRE caused by focal cortical dysplasia type II based on histopathological findings or MRI criteria. Study protocol included a 4-week baseline observation period, a 16-week dose adjustment period, and a 12-week maintenance period. Sirolimus dose titrated to target level of 5-15ng/mL.	15 patients treated with sirolimus in the 12-week maintenance period (age range 7-57 years). 13 patients previously underwent epilepsy surgery. 2 patients dropped out due non-compliance. 33% of patients had a ≥50% reduction in seizures. The median reduction rate of seizures during the maintenance phase was 25%. One patient achieved seizure freedom. All patients experienced AE, but none discontinued treatment.	Kato *et al* (2022)[334]
Case report	A 12-year-old female with epilepsy, intellectual disability, asymmetric body overgrowth and hemimegalencephaly caused by a mosaic *MTOR* mutation detected in skin cells was treated with **everolimus** for DRE. No reduction in seizure frequency was observed.	Hadouiri *et al* (2020)[335]
Case report	A 3-month-old with hemimegalencephaly was treated with **sirolimus** for DRE. She experienced a ≥50% reduction in seizures, allowing epilepsy surgery to be deferred for 2.5 months. Seizure freedom was achieved following hemispherectomy, and a somatic *MTOR* mutation was detected in brain tissue.	Xu *et al* (2019)[336]

Case report	A 3.5-month-old infant with hemimegalencephaly and a deletion involving the *NPRL3* gene was treated with **sirolimus** for DRE. There was a significant reduction in seizures but sirolimus was stopped after 3 months due to recurrent respiratory tract infections.	Vawter-Lee *et al* (2019)[337]
Case report	A 13-day-old infant with hemimegalencephaly and a deletion encompassing exon 5 of the *NPRL3* gene was treated with **sirolimus** for DRE. Sirolimus was stopped after 17 days as there was no reduction in seizures.	Chandrasekar *et al* (2021)[338]
Polyhydramnios, megalencephaly and symptomatic epilepsy (*STRADA*)		
Prospective observational case series. **Sirolimus** titrated to target serum level of 5-15ng/mL.	5 children homozygous for the *STRADA* 7.3-kb deletion were treated with sirolimus from infancy (duration of treatment 5-52 months). 4 patients achieved seizure freedom and the other had a ≥75% reduction. Compared with historical controls, sirolimus treated patients had better receptive language, and were more interactive, socially engaged and emotionally attached.	Parker *et al* (2013)[215]
***PTEN*-related neurocognitive disorder**		
Phase 2, multicentre, double-blind, randomised, placebo-controlled study of **everolimus** for neurocognitive and behavioural symptoms in patients with *PTEN* mutations (aged 5-45 years). Comprehensive neurocognitive and behavioural evaluation battery performed at baseline and 6 months.	Everolimus group (n=24), mean age 16.5 years and 25% had autism spectrum disorder. Placebo group (n=22), mean age 14.7 years and 36.3% had autism spectrum disorder. No statistically significant differences between the two groups, although several secondary outcome measures showed changes in the direction toward improvement (for example, non-verbal IQ, verbal memory, social symptoms and sensory processing). 9.1% of patients discontinued treatment due to AE (stomatitis and neutropenia).	Srivastava *et al* (2022)[327]

Abbreviations:
AE= adverse events; ASD= autism spectrum disorder; DRE= drug-resistant epilepsy; MRI= magnetic resonance imaging; PARS= Pervasive Developmental Disorders Autism Society Japan Rating Scale; SEGA= subependymal giant-cell astrocytoma; TSC= tuberous sclerosis complex.

4.3.3 Safety using mTOR inhibitors during the COVID-19 pandemic

Given their immunosuppressive effects, physicians had concerns about using mTOR inhibitors for TSC at the start of the COVID-19 pandemic. The TSC Alliance initially advised cautious use of mTOR inhibitors and to consider temporary discontinuation in patients exposed to the virus, or in cases of active COVID-19[343]. Many people with TSC reside in long-term care facilities, which are high risk settings for infection with SARS-CoV-2 and severe COVID-19[344]. Pre-existing respiratory disease was found to be a risk factor for severe COVID-19, which is relevant for those with LAM, a progressive cystic lung disease infrequently seen in women with TSC[345].

Six cases of COVID-19 in TSC patients on mTOR inhibitors were identified from the PubMed search (see **Table 4.4**)[346, 347]. All made a full recovery. Two were admitted to hospital with COVID-19 pneumonia. Everolimus was temporarily discontinued in one patient. Data from kidney and liver transplant centres did not identify an increased risk of severe COVID-19 in transplant recipients on mTOR inhibitors[348, 349].

We concluded that it was safe to initiate and continue mTOR inhibitors for TSC-related epilepsy during the pandemic. mTOR inhibitors prevent organ rejection by an immunostimulatory mechanism, via selective expansion of regulatory CD4+ T cells. Transplant recipients on mTOR inhibitors are less likely to develop cytomegalovirus infection than those treated with other immunosuppressive agents[350]. Low dose everolimus therapy reduced the annual rate of respiratory infections and enhanced the response to influenza vaccination in elderly volunteers[351].

Viruses rely on host cellular pathways for replication, utilising host transcription and translation machinery to reproduce their genome and associated proteins. Both DNA (for example, cytomegalovirus) and RNA viruses (for example, Middle East Respiratory Syndrome Coronavirus [MERS-CoV]) modulate the mTOR pathway during infection[352]. Inhibition of mTOR has been shown to suppress viral protein synthesis and interfere with virus-mediated transcription events. For example, everolimus decreased MERS-CoV replication *in vitro*[353].

The mTOR-PI3K-Akt pathway has been identified as a key signalling pathway in SARS-CoV-2 infection by proteo-transcriptomic analysis[354]. A human protein-protein interaction map of SARS-CoV-2 identified rapamycin and metformin as potential drug targets for SARS-CoV-2 due to their involvement with mTORC1 protein complex[355]. Notably, a randomized controlled trial investigating the use of metformin for preventing severe SARS-CoV-2 infection did not show any discernible benefits in terms of hypoxemia, emergency department visits, hospitalization, or COVID-19-related mortality[356].

Several viruses target host cells by inhibiting autophagy through activation of mTORC1 signaling[357]. For example, replication of Kaposi sarcoma-associated herpesvirus is facilitated by inhibition of autophagy through activation of mTORC1 signalling[358]. In contrast, several studies provide evidence that coronaviruses positively modulate host autophagic machinery while inhibiting the fusion of autophagosomes with lysosomes[359]. Therefore, autophagy modulation has emerged as an appealing treatment strategy for SARS-CoV-2 infection[357]. However, the therapeutic potential of mTOR inhibitors, which promote autophagy, remains uncertain and requires further investigation.

The immunorestorative effects of rapamycin may also enhance the efficacy of COVID-19 vaccines. Kidney transplant recipients on mTOR inhibitors had a better immune response to mRNA COVID-19 vaccines compared to patients on immunosuppressive regimens not including mTOR inhibitors, by increasing the production of vaccine-induced antibodies and stimulating the anti-SARS-CoV-2 T-cell response[360]. We encouraged our TSC patients to receive COVID-19 vaccination and did not interrupt mTOR inhibitor treatment prior to vaccination.

Table 4.4 Clinical characteristics and outcomes of people with TSC-related epilepsy on mTOR inhibitors who developed COVID-19

	Age (yrs)	Clinical features	mTORi	COVID-19 symptoms	SARS-CoV-2 RT-PCR	Hospital admission	Outcome	mTORi stopped	Reference
I	16	DRE, ID, SEGA, AML, RM	EV 3mg	Fever, cough, arthralgia	Not tested[a]	No	Full recovery	No	Peron *et al* (2020)[346]
II	8	DRE, ID, SEGA, RM	EV 3mg	Fever, diarrhoea, pneumonia	Not tested[a]	Yes	Full recovery	No	Peron *et al* (2020)[346]
III	25	DRE, ID, SEGA, RM, AML	EV 5mg	Fever, cough	Not tested[a]	No	Full recovery	No	Peron *et al* (2020)[346]
IV	6	DRE, ID, RM, AML	EV 4mg	Fever, pneumonia	Not tested[a]	No	Full recovery	Yes	Peron *et al* (2020)[346]
V	41	LAM	EV 10mg	Fever, dyspnoea	Positive	No	Full recovery	No	Baldi *et al* (2020)[347]
VI	51	LAM	SIR (not stated)	Fever, cough	Positive	Yes	Full recovery	No	Baldi *et al* (2020)[347]

Footnote
[a] Limited availability of SARS-CoV-2 PCR testing during study period. Patients either met criteria of suspect case or presented with at least two symptoms of COVID-19 or were a close contact of a confirmed case.

Abbreviations:
AML= angiomyolipoma; COVID-19= coronavirus disease 2019; DRE= drug-resistant epilepsy; EV= everolimus; ID= intellectual disability; LAM= lymphangioleiomyomatosis; mTORi= mechanistic target of rapamycin inhibitor; RM= rhabdomyoma; SEGA= subependymal giant cell astrocytoma; SIR= sirolimus; TSC= tuberous sclerosis complex.

4.4 Discussion

In this chapter, I outlined the clinical pharmacology and side-effect profile of available mTOR inhibitors, including a review on their safety of use during the COVID-19 pandemic. In comparison to sirolimus, everolimus has superior pharmacokinetics, a marginally better complication rate and more robust clinical trial experience in oncology and TSC. In mTOR inhibitor clinical trials for TSC manifestations, adverse events were very common, in particular stomatitis. Whilst stomatitis rarely led to treatment discontinuation, the emergence of stomatitis at higher everolimus doses meant many participants did not attain their target everolimus concentration[135]. Alternative mTOR inhibitor dosing strategies have been suggested in TSC that minimise drug exposure and side-effects[361]. Intermittent rapamycin dosing with 'drug holidays' maintained clinical efficacy in a mouse model of TSC[362]. Despite the paucity of information regarding mTOR inhibitor use during the pandemic, we concluded that the benefits of treating severe TSC-related DRE outweighed the risks related to immunosuppression. Emerging data from transplant populations suggest that mTOR inhibitors may enhance the immune response to mRNA COVID-19 vaccines[360].

Evidence is lacking for mTOR inhibitor treatment in children younger than two years. The EXIST-3 trial did not include TSC patients younger than two years[135]. In a small observational study of everolimus for West syndrome in infants with TSC, two out of four patients achieved electroclinical remission. No patients discontinued treatment due to adverse events after median follow-up of 13 months[340]. A retrospective study of mTOR inhibitor treatment in patients with TSC under the age of 2 years (n= 17) found everolimus to be efficacious and safe for infants with cardiac rhabdomyoma, SEGA and epilepsy[363]. However, larger prospective studies are needed to determine safety in this age category.

mTOR inhibitors display intrinsic and acquired treatment resistance in different human malignancies, and similar mechanisms may explain treatment failure in TSC. Incomplete inhibition of mTORC1 activity, failure to inhibit mTORC2 signalling, and mutations that disrupt FKBP12-rapamycin binding have been offered as potential mechanisms of resistance[364]. Brain selective ATP-competitive mTOR kinase inhibitors that target mTORC1 and mTORC2 activity are being developed to overcome resistance mechanisms, improve CNS penetration and reduce systemic side-effects.

Two novel, brain-permeable ATP-competitive mTORC1/mTORC2 inhibitors, and a dual pan-PI3K and mTORC1/mTORC2 inhibitor markedly suppressed seizures in mouse models of *Tsc1*[365]. These compounds have significantly better tolerability profiles compared to rapalogues.

After outlining the pharmacological aspects of mTOR inhibitors, I presented a scoping review on the clinical evidence supporting mTOR inhibitor use for seizures and neurological symptoms in mTORopathies. Everolimus for DRE and SEGA in TSC has class I evidence supporting efficacy and safety. Evidence for mTOR inhibitor treatment in other mTORopathies is largely drawn from small open-label prospective studies, case series and case reports (see **Table 4.3**). The disappointing results in everolimus trials for TSC-related neurocognitive and psychiatric symptoms may relate to the timing of intervention[324, 325]. It may be necessary to commence treatment earlier to observe reduced incidence of neurocognitive deficits and ASD symptoms. The modest benefits of sirolimus treatment in the FCD type II trial may have been influenced by the small sample size and short study period[334]. Furthermore, only one patient had an established genetic diagnosis (somatic *MTOR* variant), and three FCD type II diagnoses were presumptive based on radiological features. Larger studies of neuropathologically and genetically characterised FCD are needed to determine the efficacy of mTOR inhibitors for persistent seizures after epilepsy surgery in patients with FCD type II.

Applying the literature reviewed in this chapter, everolimus dosing and monitoring protocols were developed for use in our epilepsy clinic. In the next chapter, I present a retrospective study on everolimus efficacy, safety and tolerability in adult TSC patients attending three Irish epilepsy clinics.

5. A retrospective study of everolimus treatment for epilepsy in tuberous sclerosis complex: an Irish experience

5.1 Introduction

TSC is the paradigm mTORopathy, characterised by multisystem benign tumours of the brain, skin, heart, lungs and kidneys (**Table 5.1**). Neuropathological findings include cortical and subcortical tubers, subependymal nodules and SEGA. TSC is caused by LoF variants in *TSC1* or *TSC2*, with germline pathogenic variants detected in over 80% of cases[366]. Mosaic *TSC1* or *TSC2* variants were found in over half of cases lacking an identifiable germline mutation by conventional genetic testing[367].

Epilepsy is seen in 80-90% of patients with TSC who come to clinical attention[368]. However, the exact incidence of epilepsy in TSC is unknown as many people with TSC without epilepsy will not seek medical attention. Nearly two-thirds of TSC patients with epilepsy have seizure onset during the first year of life[369]. Individuals may present with a variety of seizure types including focal-onset seizures (with or without progression to bilateral tonic-clonic), infantile spasms, and generalised-onset seizures (tonic-clonic, atonic and atypical absence). DRE is common, occurring in two-thirds of patients with TSC, compared to one-third in the general epilepsy population[369].

TSC-associated neuropsychiatric disorders (TAND) are a frequent occurrence in TSC, with ID and ASD occurring in approximately half of cases[370, 371]. Mental health issues occur in two-thirds of individuals with TSC, including depression, anxiety, attention deficit hyperactivity disorder and self-injurious behaviours[372]. *TSC2* pathogenic variants predict a more severe phenotype, with a higher rate of early onset seizures, infantile spasms and developmental delay compared to patients with *TSC1* or mosaic *TSC2* variants[371, 373, 374]. *TSC1*-associated disease is more likely to be familial. The more severe phenotype seen with *TSC2* pathogenic variants may be explained by two factors. First, second-hit somatic *TSC1* variants appear to be less common than somatic *TSC2* variants[375]. Indeed, tuber counts are higher in patients with pathogenic variants in *TSC2,* which may be indicative of more frequent biallelic *TSC2* mutations[366]. Second, loss of a single *TSC2* allele appears to have a more damaging

effect on the functional activity of the hamartin-tuberin complex compared with heterozygous *TSC1* variants[257].

Historically, cortical tubers were considered the neuropathological substrate of epilepsy in TSC. However, perituberal tissues also contain pathological features that contribute to seizure generation, including dysplastic neurons, giant cells, increased axonal connectivity and dysregulated mTORC1 signalling[376]. Epilepsy surgery targeting removal of epileptogenic tubers and surrounding perituberal tissue is associated with better outcomes compared with resections that extend to the tuber margin only[377]. Moreover, tuber-free mouse models of TSC exhibit increased expression of phosphorylated S6K and spontaneous seizures, suggesting that aberrant mTORC1 signalling alone may be sufficient to generate seizures[256].

Tumours in TSC, including SEGA, AML, LAM and angiofibromas, develop due to the inactivation of both alleles of either *TSC1* or *TSC2*[378]. This follows the Knudson 'two-hit' tumour-suppressor gene model, where the first hit is a germline mutation that inactivates one copy of *TSC1* or *TSC2,* and a second somatic event inactivates the remaining wild-type allele[233]. Variable expression of hamartin and tuberin protein in different human tissues and cell types likely explain the preferential involvement of certain organs in TSC. For example, tuberin and hamartin are highly expressed in the heart during early development, followed by a dramatic reduction in expression after birth. This may explain why cardiac rhabdomyoma is commonly seen as an early manifestation of TSC[379]. Sustained high expression of tuberin and hamartin in rat CNS throughout development may provide a rationale for why epilepsy and cognitive disability are the most common manifestations in TSC[379].

LAM is the primary pulmonary manifestation of TSC, characterized by cystic lung destruction, pneumothoraces, and chylous pleural effusion[378]. Up to 80% of women with TSC have asymptomatic cystic lung disease, while symptomatic LAM, which can result in respiratory failure, occurs in 5-10% of women with TSC. Cases of biopsy-proven LAM in men are exceptionally rare[380]. The reasons behind the female predominance in LAM are not fully understood. LAM cells express oestrogen receptor-α and progesterone receptor[381], and LAM appears to progress more rapidly in premenopausal women compared to postmenopausal women, suggesting a potential

role of female sex hormones in the development and progression of LAM[380]. Moreover, there are many reports of worsening symptoms and increased pneumothorax occurrences during pregnancy, further supporting the hypothesis of hormonal influence[380]. Mouse models have also shown that oestrogen enhances the metastasis and survival of *TSC2*-deficient cells, lending further support to hormonal contributions[382]. One theory is that LAM cells originate in a female-specific organ, such as the uterus[380].

Dysregulated mTORC1 signalling results in the epilepsy, tumours and neuropsychiatric symptoms of TSC. In chapter 4, I outlined the evidence supporting the effectiveness of everolimus treatment for neurologic and neuropsychiatric manifestations of TSC. In 2017, the European Medicines Agency (EMA) approved everolimus as an adjunctive treatment for focal DRE in TSC. However, everolimus uptake has been slow in Irish epilepsy clinics, despite class I evidence supporting its efficacy.

Given the multisystem manifestations of TSC, consensus guidelines recommend an MDT approach[383]. Specialist multidisciplinary TSC clinics are considered the optimal model of care to oversee radiological surveillance of TSC tumours and monitoring of mTOR inhibitor treatment. Currently, there are over 16 specialist TSC clinics in the U.K., and over 25 in North America. There are no specialist TSC clinics in Ireland[384]. In the U.K., the decision to initiate everolimus for TSC-related epilepsy is determined by an MDT, which must include a neurologist with experience in both TSC management and therapeutic drug monitoring.

Table 5.1 The multisystem manifestations of tuberous sclerosis complex

Brain	
Epilepsy	80-90%
TSC-associated neuropsychiatric disorders	~90%
Subependymal giant-cell astrocytoma	10-15%
Kidney[385]	
Angiomyolipoma (may also involve adrenal glands and liver)	67%
Multiple renal cysts	35%
Polycystic kidney disease[a]	5%
Renal cell carcinoma	3%
Lungs[385]	
Lymphangioleiomyomatosis (almost exclusively in females) - Symptomatic lymphangioleiomyomatosis	30% of females 5-10%
Multifocal micronodular pneumocyte hyperplasia	40-60%
Heart[385]	
Rhabdomyoma (almost all regress spontaneously)	70% of infants
Eye[385]	
Astrocytic retinal hamartomas	40-50%
Skin, teeth and nails[385]	
Facial angiofibroma (previously called adenoma sebaceum)	~75%
Ungual and periungual fibroma (nails)	50-75%
Shagreen patch (thick skin patch, typically on lower back)	~50%
Hypomelanotic patches (ash leaf spots)	~90%
Gingival fibroma	20-50%
Dental enamel pits	~90%
Other rare manifestations[386]	
Scoliosis	4%
Liver and ovarian cysts	~1%
Thyroid adenoma	~1%
Pancreatic neuroendocrine tumours	0.5%

Footnote:

[a] The *TSC2* gene lies adjacent to the *PKD1* gene. Pathogenic variants in *PKD1* cause autosomal dominant polycystic kidney disease. Deletion mutations involving *TSC2* and *PKD1* cause TSC with severe renal cystic disease[387].

Here I present the Irish experience using everolimus for TSC-related DRE at three tertiary epilepsy centres. The aims of this chapter are:

a) To develop everolimus dosing and monitoring guidelines for use at the epilepsy clinic.
b) To estimate the number of adult TSC patients eligible for treatment with everolimus attending three Irish epilepsy clinics.
c) To study the efficacy, safety and tolerability of everolimus treatment for TSC-related DRE in patients attending three Irish epilepsy clinics.

5.2 Methods

5.2.1 Everolimus dosing and monitoring guidelines for TSC-related epilepsy

Everolimus dosing and monitoring protocols for TSC-related epilepsy were developed using the literature reviewed in chapter 4 (see **Table 5.2**). Data from prospective clinical trials of everolimus, post-approval 'real-world' studies, and expert opinion reviews were used to develop protocols. To increase awareness and knowledge on the clinical utility of everolimus for TSC-related epilepsy, I delivered a presentation to the Beaumont Hospital epilepsy group on epilepsy management and tumour surveillance in TSC. I arranged virtual meetings with the epilepsy teams in St. James's Hospital and Cork University Hospital to present the everolimus dosing and monitoring guidelines. To increase awareness within the Irish neurology community, I presented an illustrative TSC clinical case on everolimus treatment at the weekly national neuroscience meeting.

Table 5.2 Everolimus dosing and monitoring protocols

Everolimus (Afinitor®) available in 2.5mg, 5mg and 10mg tablet and oral dispersible preparations. Must be prescribed on a High Tech Prescription.
Check full blood count, renal function tests, liver function tests, fasting lipid profile and fasting glucose prior to starting everolimus.

a) The everolimus starting dose is 8mg/m^2/day in patients taking CYP3A4 or P-glycoprotein inducers (carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone, clobazam, topiramate) or 5mg/m^2/day in patients not taking CYP3A4 or P-glycoprotein inducers. The starting dose is rounded to a dose divisible by 2.5mg.
b) Everolimus is titrated to a target trough level of 5-15 ng/mL. An everolimus level should be checked within eight weeks of starting treatment or after a dose change. Blood samples are taken in EDTA (ethylenediaminetetraacetic acid) blood collection tubes (collection tube used for full blood count). Ideally, the blood sample should be taken one hour before the next everolimus dose. Irish hospitals send everolimus level blood samples to the Harefield Hospital Immunosuppression Monitoring Service (IMS) Laboratory in Uxbridge, London. Samples are analysed by mass spectrometry.
c) Full blood count, renal function tests, liver function tests, fasting lipid profile, fasting glucose and serum everolimus level should be checked every six months on treatment.
d) When titrating the everolimus dose the following equation can be applied:

   New dose= current dose x (target concentration/current concentration).

e) **Common everolimus side-effects.**
   - Stomatitis 35% (severe in 2.5% of patients exposed).
   - Fever 35%.
   - Raised cholesterol 25%.
   - Irregular periods in women 25%.
   - Vomiting or diarrhoea 20%.
   - Nasopharyngitis and upper respiratory tract infection 15%.
   - Acne-like skin rash 15%.
   - High blood sugar 10%.
   - Abnormalities on full blood count 10% (severe cytopenia <2%).
   - Severe pneumonitis <1%.

   The incidence of side-effects reduces over time.

f) Management of side-effects.

- Stomatitis
    - Good oral hygiene, soft toothbrush, children's toothpaste, saline rinses after meals.
    - Avoid alcohol-based mouthwashes, spicy and acidic foods.
    - Mouthwash options: KIN or BMX (both can be swallowed).
    - If mouth dry- Glandesene or Bioxtra gel.
    - If severe hold everolimus for 3-5 days, and then reduce dose by 2.5mg. Systemic steroids also an option.
- Infection or pyrexia or pneumonitis
    - Interrupt treatment until symptoms resolve.
    - If recurrent consider dose reduction by 2.5mg.
- High cholesterol or hyperglycaemia
    - Dietary advice and exercise.
    - If cholesterol levels remain high, consider everolimus dose reduction by 2.5mg or addition of statin.
    - Referral to endocrinology for new-onset diabetes mellitus.
- Acne-like rash
    - Referral to dermatology if painful or causing psychological distress.

g) Contraception

Women of childbearing potential advised to use a highly effective method of contraception for duration of treatment and 8 weeks after cessation of treatment, as the effects of mTOR inhibitors on the developing foetus are unknown.

References

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5.2.2 TSC patients with epilepsy eligible for everolimus treatment

A list of all TSC patients attending the Beaumont Hospital epilepsy clinics was retrieved from the epilepsy EPR using the following search terms: "tuberous sclerosis" *OR* "genetic aetiology" *OR* "neurocutaneous syndrome". The neurologist running the epilepsy clinic at Cork University Hospital (Professor Daniel Costello) keeps a database of all patients with TSC attending his clinic. Similarly, an epilepsy advanced nurse practitioner working at St. James's Hospital (Ms Claire Behan) maintains a database of TSC patients attending Professor Colin Doherty's epilepsy clinic.

After collating data from the three sources, a list of 54 adult TSC patients with epilepsy was generated. Demographic and clinical characteristics were accessible for Beaumont Hospital and St. James's Hospital patients through the epilepsy EPR. Relevant clinical data was extracted from clinic letters for Cork University Hospital patients. Data collected included age, sex, seizure type and frequency, age of seizure onset, current and prior ASMs, VNS or epilepsy surgery, family history, multisystem manifestations, TAND, genotype, and current or prior use of everolimus. TSC patients with DRE were considered eligible for everolimus treatment. DRE was defined as failure to control seizures after *"adequate trials of two tolerated and appropriately chosen ASM schedules*"[61].

5.2.3 Retrospective study on the efficacy, safety and tolerability of everolimus for TSC-related DRE in patients attending three Irish epilepsy clinics

Patients with TSC who were eligible for everolimus treatment were offered appointments at epilepsy clinic to discuss the option of an everolimus trial. Patients with TSC attended with their families and/or carers, as most eligible patients had severe ID. The benefits and risks associated with everolimus therapy were thoroughly explained to patients and carers, and an information sheet was provided (see **Appendix 4**). If patients and/or carers consented to everolimus treatment, baseline blood tests were sent (see **Table 5.2**). Advice regarding oral hygiene and diet, and a prescription for Kin (0.12% chlorhexidine digluconate) gingival mouthwash were provided to reduce the risk of stomatitis. Everolimus was initiated at 8mg/m^2 daily in patients taking concomitant CYP3A4 inducers and 5mg/m^2 daily in patients not taking

CYP3A4 inducers[308]. The everolimus dose was titrated to a target blood trough concentration of 5-15ng/mL. Everolimus serum concentrations were checked within eight weeks of treatment initiation or after a dose change, and then every six months. Blood tests arranged at baseline were repeated every six months. Virtual consultations were arranged for some patients and family members attending the epilepsy clinics in Cork University Hospital and St. James's Hospital.

I performed a retrospective study of all patients treated with everolimus for TSC-related DRE attending the three epilepsy clinics. Baseline monthly seizure frequency was estimated retrospectively based on the seizure frequency recorded in the EPR, and on patient or carer reports over the three months prior to everolimus initiation. The primary outcome measure was change in monthly seizure frequency compared with baseline. Seizure freedom was defined as "*freedom from seizures for a minimum of three times the longest preintervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever is longer*" [61]. Treatment responders were defined as those with a greater than 50% seizure frequency reduction. Treatment-emergent adverse events were recorded using a structured questionnaire utilised in a study of everolimus for infants with TSC and graded according to the Common Terminology Criteria for adverse events (see **Appendix 5**) [340]. Concomitant ASM adjustments and reasons for everolimus discontinuation were also analysed. Additional benefits unrelated to seizure control (for example, improved skin or neuropsychiatric symptoms) were recorded.

This study was a retrospective analysis of existing clinical data, so ethics committee review and patient consent were not required. Data is summarised descriptively.

5.3 Results

5.3.1 TSC epilepsy patients eligible for treatment with everolimus

Fifty-four TSC patients with epilepsy were attending the three tertiary epilepsy clinics (22 patients in Beaumont Hospital, 8 patients in Cork University Hospital and 24 patients in St. James's Hospital). Of these, 31 had DRE (57.4% of the cohort) and were eligible for treatment with everolimus. The remaining 23 patients had inactive epilepsy. Two patients were already taking everolimus for TSC-related DRE, whilst another patient with DRE was taking everolimus for renal angiomyolipoma (AML). One patient with TSC-related DRE had previously trialled everolimus but stopped treatment due to side-effects. Everolimus remained a treatment option for 27 TSC patients with DRE (see **Figure 5.1**).

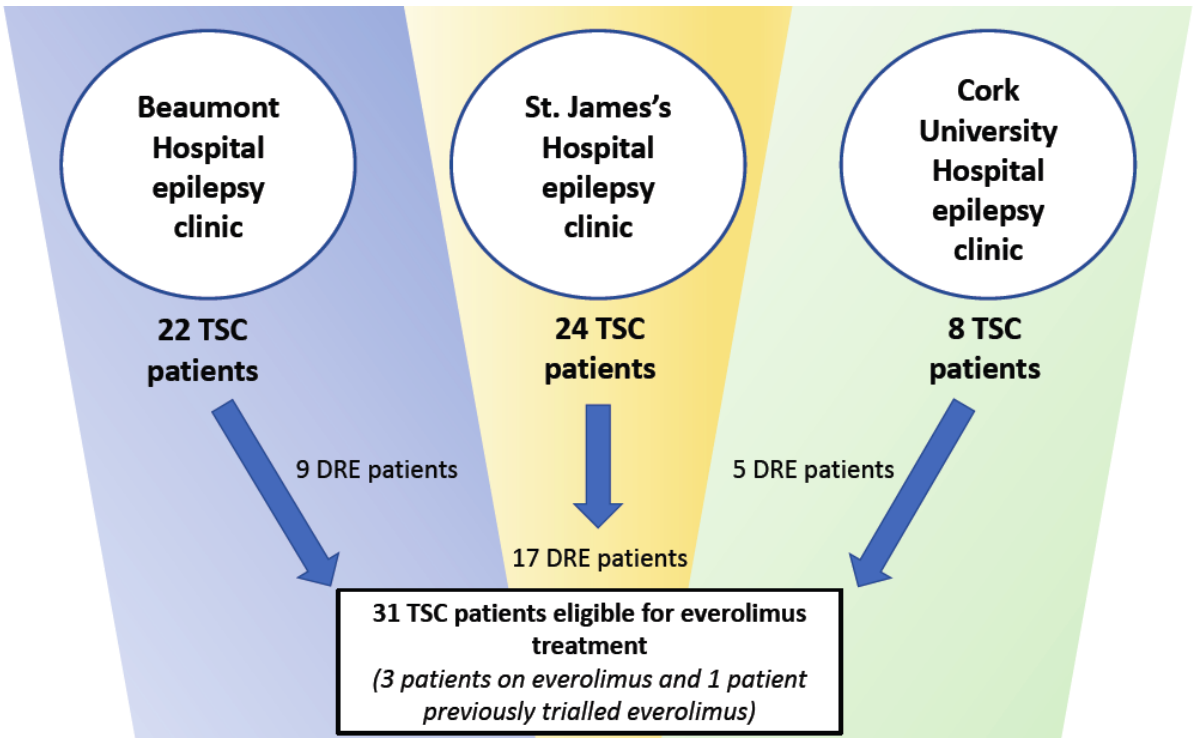

Figure 5.1 TSC patients with drug-resistant epilepsy attending three Irish epilepsy clinics

5.3.2 Retrospective study on the efficacy, safety and tolerability of everolimus for TSC-related DRE in patients attending three Irish epilepsy clinics

Nine additional TSC patients started everolimus treatment for DRE during the study period. In total, thirteen patients with TSC-related DRE have been treated with everolimus, representing 42% of the eligible population. Another patient with SEGA and inactive epilepsy started everolimus for SEGA tumour volume reduction. Baseline demographics and clinical characteristics are outlined in **Table 5.3**. The cohort had a median age of 32 years (range 17-54 years). Over three-quarters of patients had active bilateral tonic-clonic seizures. Six patients (46.2%) had combined generalised and focal epilepsy[14]. This diagnosis was based on vEEG recordings demonstrating both generalised-onset and focal-onset seizures.

This cohort had highly refractory epilepsy. The median number of concomitant ASMs at the time of everolimus initiation was four (range 1-5). The cohort had previously failed a median of four ASMs (range 1-12). Two patients had VNS. Nine patients had pathogenic variants in *TSC2* (3 splice-site, 3 deletion, 2 in-frame microdeletion and 1 nonsense) and three had *TSC1* pathogenic variants (1 frameshift microduplication, 1 frameshift microdeletion and 1 splice-site). One patient never had genetic testing. She was diagnosed with TSC based on clinical criteria. Almost 70% of patients had TAND, including eight patients with ID and/or ASD. Four patients had prior neurosurgery for SEGA, and nine patients had AML under radiological surveillance (see **Table 5.3**).

Of the 13 TSC patients treated with everolimus for DRE, eight remain on treatment. Two patients stopped treatment due to lack of efficacy (one patient also had recurrent severe stomatitis), two patients stopped because of severe stomatitis, and one patient stopped to become pregnant. The female patient who stopped everolimus to become pregnant had been seizure-free for five months and was keen to restart everolimus after the pregnancy. The median duration of treatment was 24 months (range 5-95 months). The median everolimus dose was 10mg/day (range 10-15mg). The mean serum everolimus level was 7.41ng/mL (range 5.8-12.4ng/mL).

Table 5.3 Baseline characteristics of TSC drug-resistant epilepsy patients treated with everolimus

n=13	
Median age, years (range)	32 (17-54)
Male, n (%)	7 (53.8)
Seizure types, n (%)	
Focal aware	2 (15.4)
Focal impaired awareness	10 (76.9)
Focal to bilateral tonic-clonic	10 (76.9)
Generalised (tonic, atonic, atypical absence)	6 (46.2)
Median seizure frequency per month (range)	22 (3-120)
Median age of first seizure, year (range)	1 (0.125-10)
Median number of current ASMs, n (range)	4 (1-5)
Median number of prior ASMs, n (range)	4 (1-12)
Vagus nerve stimulation or epilepsy surgery, n (%)	
Vagus nerve stimulation	2 (15.4)
Epilepsy surgery	0
Genotype, n (%)	
TSC1 pathogenic variant	3 (23.1)
TSC2 pathogenic variant	9 (69.2)
Clinical diagnosis of TSC (genetic testing not done)	1 (7.7)
TSC-associated neuropsychiatric disorders, n (%)	9 (69.2)
Intellectual disability	8 (61.5)
Autism-spectrum disorder	5 (38.5)
Depression or anxiety	2 (15.4)
Psychogenic non-epileptic seizures	1 (7.7)
Multisystem manifestations, n (%)	
Angiomyolipoma	9 (69.2)
Rhabdomyoma	2 (15.4)
Lymphangioleiomyomatosis	1 (7.7)
Subependymal giant-cell astrocytoma	4 (30.8)
Dermatological	11 (84.6)

Among patients continuing on everolimus treatment, three had a greater than 75% reduction in seizure frequency and three had a 50-75% reduction. Overall, seven patients (53.8%) benefited from everolimus treatment, including the patient who stopped treatment to become pregnant (see **Figure 5.2**). Due to the small sample size, it was not feasible to determine predictors of treatment response. Two out of three (66.66%) patients with *TSC1* variants had a favourable response to everolimus treatment, while five out of nine (55.55%) patients with *TSC2* variants benefited from treatment. Six patients (46.2%) developed stomatitis, including one who required antibiotic treatment for a gingival abscess. Six patients (46.2%) developed hypercholesterolemia, of whom one has started statin therapy. None of patients treated with everolimus experienced an increase in infections, and no cytopenias were detected on surveillance blood testing.

Improvements in dermatological manifestations of TSC were noted in two patients. Whether coincidental or otherwise, two patients with TAND had improved psychiatric and behavioural symptoms on everolimus treatment. Treatment outcomes and complications are summarised in **Table 5.4**. Two case histories are presented to illustrate the potential therapeutic benefit of everolimus for refractory epilepsy in TSC. A brief overview of the TSC patient treated with everolimus for SEGA is also presented.

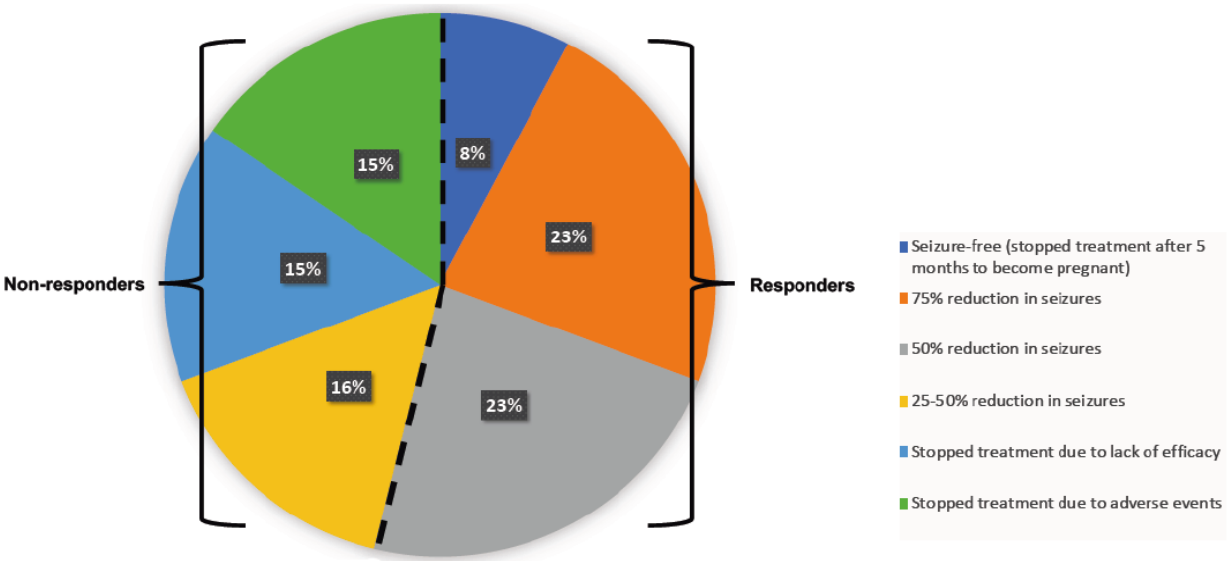

Figure 5.2 Outcomes of 13 patients with TSC-related drug-resistant epilepsy treated with everolimus

Table 5.4 Summary of treatment outcomes and complications in TSC patients treated with everolimus

n	Genotype	Baseline seizure frequency	Duration of treatment	Maximum everolimus dose (level)	Outcome	Adverse events
1	*TSC2* Splice-site	120 per month	95 months	15mg (7.1ng/mL)	>50% seizure reduction	High cholesterol (now on statin)
2	*TSC2* Splice-site	12 per month	37 months	12.5mg (7.8ng/mL)	>50% seizure reduction, reduced anxiety	High cholesterol
3[a]	*TSC2* Splice-site	3 per month	67 months	10mg *(No trough level obtained)*	>75% seizure reduction, AML stable	High cholesterol
4	*TSC2* Deletion (Exon 14-20)	8 per month	27 months	10mg (8.0 ng/ml)	>75% seizure reduction, improved behaviour	High cholesterol
5	*TSC2* Deletion (Half exon 28 & adjacent intron)	80 per month	25 months	12.5mg (5.7 ng/mL)	>50% seizure reduction, improved skin	High cholesterol
6	*TSC2* Deletion (Exon 16)	22 per month	25 months	12.5mg (5.8 ng/mL)	25-50% seizure reduction	Nil
7	*TSC2* Nonsense	40 per month	24 months	10mg *(No trough level obtained)*	25-50% seizure reduction, improved skin	Moderate stomatitis
8	*TSC1* Frameshift microdeletion	30 per month	18 months	15mg (6.6ng/mL)	No seizure reduction *(stopped due to lack of efficacy)*	Severe stomatitis (abscess), skin pustules
9	*TSC1* Splice-site	10 per month	17 months	12.5mg (12.4 ng/mL	>75% seizure reduction	Mild stomatitis
10	*TSC2* In-frame microdeletion	12 per month	7 months	10mg *(No trough level obtained)*	>50% seizure reduction *(stopped due to stomatitis)*	Severe stomatitis
11	*TSC1* Frameshift micro-duplication	6 per month	5 months	10mg *(No trough level obtained)*	Seizure-free *(stopped to become pregnant)*	Nil
12	*TSC2* In-frame microdeletion	120 per month	5 months	10mg (5.9 ng/mL)	No seizure reduction *(stopped due to lack of efficacy)*	Moderate stomatitis
13	Not known (clinical diagnosis of TSC)	80 per month	5 months	10mg *(No trough level obtained)*	>50% seizure reduction *(stopped due to stomatitis)*	Severe stomatitis

Footnotes: [a] Treated with everolimus for angiomyolipoma but also experienced reduced seizures.

5.3.3 Case history 1

This 40-year-old woman with TSC resides in a long-term care facility. She has severe ID and ASD, often exhibiting self-injurious behaviours (for example, head-banging). Her seizures began at the age of three years. Her seizure types include focal non-motor seizures with impaired awareness, focal to bilateral tonic-clonic seizures and epileptic spasms. Before the addition of everolimus, she had 8-10 seizures per month despite treatment with five ASMs (clobazam, eslicarbazepine, perampanel, rufinamide and sodium valproate). She had previously failed six ASMs (brivaracetam, carbamazepine, clonazepam, lamotrigine, levetiracetam and zonisamide). Recurrent lower respiratory tract infections often provoked seizure clusters that necessitated admission to her local hospital. Her brain imaging showed cortical and subcortical tubers, and subependymal nodules. She has AML under radiological surveillance. Genetic testing identified a heterozygous intragenic deletion of exons 14-20 in the *TSC2* gene.

In September 2020 she commenced everolimus 10mg daily. After 27 months on treatment, she experienced a greater than 75% reduction in seizure frequency. Her most recent serum everolimus level was 8.0ng/mL. There have been occasional seizure-free months and she has not required hospital admission for seizure clusters since everolimus was added. An attempt to withdraw sodium valproate was aborted due to seizure worsening. Her carers report that self-injurious behaviours have reduced since the introduction of everolimus. She continues to have intermittent chest infections, but their frequency has not increased since starting everolimus. Her total cholesterol level has increased to 8.2mmol/L. This level likely warrants pharmacological intervention but her family and carers have opted to trial dietary adjustments prior to initiating statin therapy.

5.3.4 Case history 2

A 17-year-old man with TSC commenced everolimus in his fifth year of secondary school. He has no learning difficulties and aimed to study engineering in university. He began having seizures aged 10 years. His seizures are stereotyped episodes comprising an auditory aura, followed by automatisms (lip-smacking, repetitive hand clenching and gasping) and loss of awareness. Prior to everolimus, he had around 10 seizures per month. His seizures caused significant post-ictal fatigue and many

missed school days. He was treated with carbamazepine slow release 200mg twice daily and zonisamide 250mg twice daily. He was previously treated with lacosamide. He required surgical resection of a SEGA tumour aged nine years, which prompted the diagnosis of TSC. An asymptomatic rhabdomyoma was detected on echocardiogram. He inherited a *TSC1* splice-site variant from his father, who only exhibits dermatological manifestations of TSC. His only brother also has TSC and underwent SEGA tumour resection aged three years.

He started everolimus 7.5mg daily, with up-titration to 12.5mg daily over six months. After 17 months on treatment, he experienced a greater than 75% reduction in seizures. Now he reports shorter and less “intense” seizures. Significantly, he missed fewer school days, and secured a place on an engineering course in an Irish university. His most recent serum everolimus concentration was 12.4ng/mL. He reported no significant adverse events, apart from mild stomatitis. Surveillance blood testing was unremarkable.

5.3.5 Case history 3

This 20-year-old man has a clinical diagnosis of TSC. He has severe ID and inactive epilepsy. His epilepsy is controlled on levetiracetam, lamotrigine and sodium valproate. A 15mm SEGA tumour was detected on a surveillance MRI brain in 2019. He was asymptomatic and his neurological examination revealed no signs of raised intracranial pressure. He commenced everolimus 10mg daily, and after three years on treatment the SEGA diameter decreased to 12mm (see **Figure 5.3**). There have been no treatment-emergent adverse events.

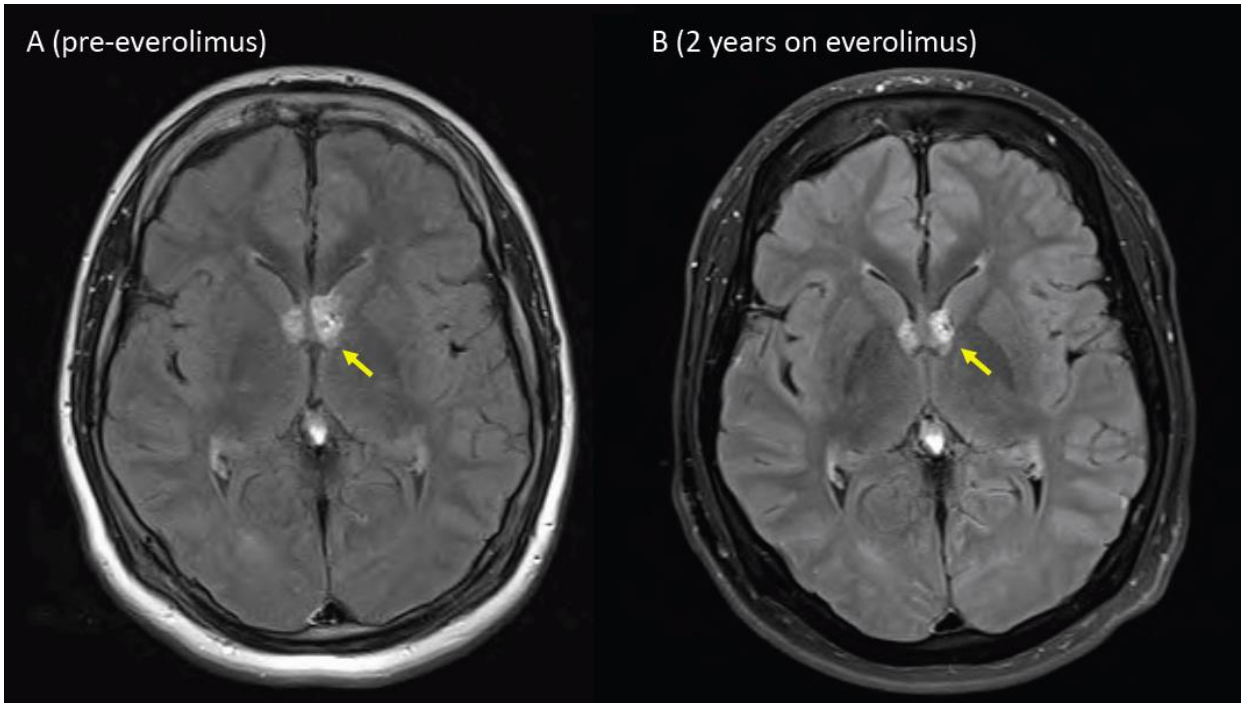

Figure 5.3 MRI brain of TSC patient treated with everolimus for subependymal giant-cell astrocytoma.

Image **A** demonstrates bilateral subependymal giant-cell astrocytomas on an axial FLAIR sequence. The larger left-sided lesion is highlighted by the yellow arrow (15mm maximal diameter). This MRI brain was performed before starting everolimus treatment. The second MRI brain **(B)** was performed two years after starting everolimus treatment. Both lesions reduced in size. The larger lesion highlighted by the yellow arrow was 12mm on follow-up imaging.

5.4 Discussion

In this chapter I report the experience of three tertiary epilepsy centres using everolimus in adult TSC patients with highly active and refractory epilepsy. More than half of treated patients experienced meaningful seizure outcomes. These included three patients with a greater than 75% seizure frequency reduction, three patients with a 50-75% reduction, and one patient who achieved seizure freedom for five months before stopping everolimus to become pregnant. A greater than 50% seizure frequency reduction was considered a meaningful outcome, given the epilepsy severity in the cohort. Many patients had exhausted available therapeutic options and continued ASM trials were unlikely to yield seizure remission. The disease-modifying potential of everolimus was evident in patients who experienced both seizure frequency reductions and improvements in other TSC-related clinical features, including dermatological, renal and neuropsychiatric manifestations.

Treatment-emergent adverse events were common, particularly stomatitis and hypercholesterolemia. Severe stomatitis contributed to treatment discontinuation in three patients, despite our best efforts with mouthwashes and everolimus dose reduction. Tolerability issues were apparent in the clinical trials, limiting attainment of target serum everolimus levels in many participants[135]. Epilepsy specialists lack expertise managing stomatitis. TSC patients on everolimus would benefit from management at specialist TSC multidisciplinary clinics, where physicians and nurse specialists have experience using mTOR inhibitors.

In some patients, lack of efficacy may be due to their older age and the longstanding intractable nature of their epilepsy. In the EXIST-3 trial, participants had a median age of 10 years, and just under half had failed less than six ASMs before trialling everolimus[135]. Irish patients treated with everolimus had a median age of 32 years, and over 60% had trialled six or more ASMs. In a retrospective study of 45 adult TSC patients with epilepsy attending seven German centres, one-third of patients treated with everolimus had a greater than 50% reduction in seizure frequency[388]. The mean age of the German TSC cohort was 31.6 years, similar to Irish TSC patients on everolimus. Findings from the present study and the German retrospective analysis indicate that TSC patients of all ages can benefit from everolimus treatment.

Epileptogenesis in TSC appears to be a progressive process. Cyst-like tubers are seen in almost half TSC patients and are associated with epileptic spasms and DRE[389]. Serial MRI showed that cyst-like tubers can increase in size and number. Moreover, they have a similar neuropathological appearance to some neurodegenerative white matter disorders, such as megalencephalic leukoencephalopathy with subcortical cysts[389]. Interictal epileptiform discharges herald impending epilepsy in seizure naïve infants with TSC[390]. The EPISTOP study demonstrated that treatment with vigabatrin at the onset of epileptiform abnormalities on EEG, delayed the onset of seizures, reduced the severity of epilepsy, and reduced the frequency of neurodevelopmental delay, compared with patients who received vigabatrin after their first seizure[391].

As pre-emptive vigabatrin improved epilepsy outcomes in TSC infants with abnormal EEG, additional benefits may be attainable with early mTOR inhibitor therapy in seizure naïve TSC patients. Evidence suggests that early dynamic mTOR-dependent processes during development influence epileptogenesis in TSC. Therefore, early inhibition of mTOR hyperactivation has the potential to prevent the development of epilepsy in TSC. Indeed, early rapamycin treatment prevented severe neurological phenotypes and epilepsy in rodent models of TSC[256, 257]. A Chinese registry-based study examined the impact of sirolimus treatment for rhabdomyoma, AML or SEGA before the onset of seizures on the later development of epilepsy. Patients treated with sirolimus before the age of 12 months had delayed onset of seizures compared to matched controls. Furthermore, TSC patients treated with sirolimus in the first year of life were less likely to develop epileptic spasms and DRE[392].

Large prospective studies are needed to determine safety of mTOR inhibitors in infants. In a mouse model of TSC, prenatal rapamycin treatment led to hippocampus-dependent memory and learning deficits, which were not observed in mice treated postnatally[393]. This suggests that early mTORC1 inhibition has the potential to alter pivotal anatomical structures involved in cognition during neurodevelopment. An optimum ‘time window’ for early treatment, that maximises epilepsy and cognitive outcomes without impacting neurodevelopment remains to be elucidated. Early or preventative treatment trials should also take into account the optimal duration of treatment, considering that long-term treatment may not be required to prevent

epileptogenesis. Furthermore, the potential long-term effects on growth and immunity in young children are largely unknown. Controlled trials usually have a limited duration of a few years, which may not be sufficient to investigate these questions comprehensively. Prospective cohort studies may offer valuable insights into the long-term effects of treatment.

The use of prophylactic everolimus after surgical removal of TSC-related tumours is a therapeutic strategy that has yet to be explored. TSC 'breaks the rules' of typical epilepsy surgery, as most patients present with multiple seizure foci. However, emerging surgical strategies, such as SEEG and MRI-guided laser interstitial thermal therapy, enable the identification and treatment of multiple seizure foci within complex epileptogenic networks[394]. Since achieving seizure freedom through epilepsy surgery is often unrealistic for patients with multiple tubers, prophylactic everolimus treatment following epilepsy surgery may lead to improved outcomes. In a pilot study investigating the impact of everolimus treatment 7-21 days before epilepsy surgery in TSC patients, no safety concerns or adverse outcomes related to wound healing were identifed[395]. However, the effects of everolimus treatment after surgery in TSC have not yet been studied. Prophylactic everolimus treatment may be beneficial following SEGA or AML resection, as there is a small risk of tumour recurrence after surgery[378].

Prenatal diagnosis of TSC is possible with ultrasonographic detection of cardiac rhabdomyoma or SEGA. Neonates with TSC diagnosed prenatally and familial TSC cases could be targeted for recruitment in pre-emptive mTOR inhibitor studies. Kingsmore *et al* demonstrated the value of early high-throughput sequencing in seriously ill infants with diseases of unknown aetiology[396]. A genetic diagnosis was established in 23-43% of cases, of whom one-third had their treatment changed and one-fifth avoided major morbidity following an early molecular diagnosis[396, 397]. An early molecular TSC diagnosis would facilitate more accurate prognostication, especially in infants with pathogenic *TSC2* variants for whom a more severe phenotype can be predicted[373]. Those with *TSC2* mutations could be prioritised for serial EEG monitoring and considered for early intervention with disease-modifying therapies. Ultimately, gene therapy may be a viable alternative to rapalogues, with the potential to improve long-term outcomes in TSC if administered early. In a mouse model of *TSC2* with prominent SEGA-like lesions, intravenous injection of an AAV

vector carrying a condensed form of tuberin led to reduced tumour volumes and improved survival[398].

A pattern of reduced seizures during the initial months of treatment followed by return to pre-everolimus seizure frequencies was observed in three patients. The three patients had previously experienced the so-called “honeymoon effect” on other ASMs[399]. Three patients with suboptimal responses to everolimus were subsequently treated with cannabidiol (CBD). CBD is an approved treatment for epilepsy in TSC, Dravet syndrome and Lennox-Gastaut syndrome. A phase 3 randomised, placebo-controlled trial supports the efficacy of CBD for DRE in TSC[400]. Two of the three TSC patients treated with CBD have experienced improved seizure control. CBD has been shown to increase serum levels of everolimus and sirolimus. No patients in the phase 3 CBD trial were taking concomitant mTOR inhibitors, so potential synergistic or toxic effects are unknown[400, 401]. Another TSC patient who derived no benefit from everolimus experienced a greater than 50% seizure frequency reduction on cenobamate, the most recently approved ASM for focal-onset DRE =[74].

Several methodological limitations warrant mentioning. First, as a retrospective study, everolimus treatment was neither randomised nor blinded. There was no standardised format for recording seizure frequency in the medical record. We relied on the EPR and patient or carer reports, which may underestimate the actual seizure frequency. Second, the study was limited by the small sample size. Third, the 'real-world' orientation of the study meant patients had heterogeneous treatment regimens, including ASM combinations that were introduced and withdrawn at different time points. Therefore, attributing a clinical response to an individual intervention was challenging. Despite these limitations, our findings highlight a significant benefit in some patients with highly active and refractory epilepsy, even if few patients achieved seizure freedom.

In conclusion, this retrospective analysis illustrates the potential of everolimus as a precision medicine in adult TSC patients with severe refractory epilepsy. Over half achieved meaningful treatment outcomes, including reduced seizure rates, fewer hospital admissions, and improved neuropsychiatric symptoms. Stomatitis is a common dose-limiting complication, requiring thorough pretreatment counselling on

prevention strategies and accessible lines of support throughout treatment. Everolimus is a viable treatment option for DRE in TSC, alongside CBD, neuromodulation therapies and epilepsy surgery. Pre-emptive mTOR inhibitor therapy is an appealing strategy in TSC. A prospective, placebo-controlled trial of early sirolimus to prevent or delay seizure onset in TSC infants is underway (NCT05104983).

6. Deep phenotyping study of Irish patients with GATOR1-related epilepsies

6.1 Introduction

Heterozygous pathogenic variants in genes encoding the GATOR1 subcomplexes *DEPDC5*, *NPRL2* and *NPRL3* are a major cause of focal epilepsy and represent a distinct subset of mTORopathies, functionally subclassified as 'GATORopathies'[229]. *DEPDC5* mutations account for 83% of all GATOR1-related epilepsies, while the remaining 17% is made up of *NPRL2* (6%) and *NPRL3* (11%) variants[127]. The longer length of the *DEPDC5* transcript (5551 base pairs [bp]) compared with *NPRL2* (1700 bp) and *NPRL3* (2881 bp), and the more recent discovery of epilepsy-causative *NPRL2* and *NPRL3* variants are potential reasons for the increased frequency of *DEPDC5*-associated epilepsies[127].

Pathogenic or likely pathogenic variants in *DEPDC5, NPRL2* and *NPRL3* were identified in 8-11% of patients in focal epilepsy cohorts, mostly comprised of familial non-lesional cases (see **Table 6.1**)[130, 131, 402]. These prevalence rates likely overestimate the true frequency of GATOR1-related epilepsies, as the cohorts were enriched with familial focal epilepsy cases. Large international collaborative studies have demonstrated the contribution of ultra-rare variants in known epilepsy genes to common epilepsies, like non-acquired focal epilepsy. In a study by the Epi4K and Epilepsy Phenome/Genome Project, approximately 3% of patients with familial non-lesional focal epilepsy had deleterious *DEPDC5* variants[140]. The Epi25 collaborative study found damaging *DEPDC5* variants in approximately 0.4% of patients with sporadic non-lesional focal epilepsy[403].

The GATORopathies have a 'brain only' phenotype, encompassing a broad spectrum of non-lesional and lesional epilepsies[127, 129-131]. FFEVF is the paradigmatic phenotype characterised by intrafamilial variability, where seizure patterns and EEG localisations differ among affected family members[129]. Affected individuals harbouring the same GATOR1 variant may have normal brain imaging or FCD type II[131, 235].

GATORopathies display incomplete penetrance, with variants dominantly inherited from asymptomatic parents in approximately 60% of cases[127, 404]. Germline GATOR1 variants have been identified in individuals and families with nocturnal frontal lobe epilepsy[127, 130, 132, 133, 405], temporal lobe epilepsy[127, 130, 132], epilepsy with auditory features[406], epileptic spasms[127, 407], and self-limited epilepsy with centrotemporal spikes[408]. Seizures occur predominantly from sleep in almost half with GATOR1-related epilepsies[127]. Seizures usually emerge in childhood or adolescence, but the age of first seizure has ranged from the first days of life to older than 50 years (see **Table 6.1**)[127, 129, 131, 402]. Importantly, over half of patients with GATOR1-related epilepsies have DRE[127].

MCD was identified in over 20% of reported GATOR1-related epilepsies, most commonly FCD type II[90, 91, 127, 131, 134, 142]. FCD type I[127, 142, 235], BOSD[134, 158, 240], HME[142, 242], polymicrogyria[130], and subcortical band heterotopia[240] have also been observed in GATORopathies (see **Table 6.1**). Cognitive deficits are seen in approximately half of affected individuals, and psychiatric disorders observed in over 40% of cases[127]. Co-morbid ASD is seen in around 10% of GATOR1-related epilepsies[127]. An ASD only phenotype has also been reported in patients with *DEPDC5* pathogenic variants[127, 409]. No distinct phenotypic characteristics have been identified when comparing individuals with *DEPDC5*, *NPRL2* or *NPRL3* variants, although FCD has rarely been reported in patients with *NPRL2* mutations[127, 130, 131]. Second-hit somatic variants in *DEPDC5* have been detected in resected FCD tissue from patients with germline *DEPDC5* variants, explaining how focal lesions develop in patients with germline mutations affecting all cells in the body[90, 91, 142, 143, 234, 235].

Table 6.1 A summary of the epidemiological, genetic and phenotypic characteristics of GATOR1-related epilepsies

Clinical characteristics	
Prevalence in focal epilepsy cohorts	
Focal epilepsy cohort (n=404), mostly comprised of familial non-lesional cases[130]	9.4%
Focal epilepsy cohort (n=93), mostly comprised of familial non-lesional cases[131]	11%
Non-lesional focal epilepsy cohort (n=112, 66% sporadic)[402]	8%
Familial non-lesional focal epilepsy cohort (n=525)[140]	2.6%
Sporadic non-lesional focal epilepsy cohort (n=7489)[403]	0.4%
[a] Mean age of seizure onset (range)[127, 129, 131-134, 278, 402, 405-408, 410]	9 yrs (0-52 yrs)
[b] Distribution of epilepsy phenotypes	
Nocturnal frontal lobe epilepsy[127, 129, 130, 132, 133, 235, 240, 405]	42%
Temporal lobe epilepsy (including epilepsy with auditory features)[127, 129, 130, 402, 406]	7%
Familial focal epilepsy with variable foci[129, 131, 132, 235, 402, 410]	11%
[c] *Other focal epilepsies*[127, 130-132, 134, 142, 235, 278, 402, 408, 410]	26%
Epileptic spasms[127, 407]	6%
Generalised epilepsy[127, 142]	4%
Self-limited epilepsy with centrotemporal spikes[408]	3%
Complex febrile seizures[127]	1%
[d] Frequency of malformations of cortical development[127, 129, 131-134, 142, 235, 240, 278, 405-408, 410]	23%
Frequency of drug-resistant epilepsy[127]	54%
[e] Frequency of sudden unexpected death in epilepsy in families with GATOR1 pathogenic variants[127]	9.3%
Frequency of cognitive co-morbidities[127]	46%
Autism spectrum disorder	9%
Frequency of psychiatric co-morbidities[127]	43%
Oppositional disorder	18%
Attention deficit hyperactivity disorder	15%
Depression or anxiety	8%
Distribution of GATOR1 variants[127]	
DEPDC5	83%
NPRL2	11%
NPRL3	6%

Mode of inheritance[127]	
De novo	4%
Inherited	96%
Frequency of mutation types[127]	
Loss-of-function (stop-gain, frameshift)	67%
Missense	27%
Splice-region	4%
Penetrance[127, 129]	66%

Footnotes:
[a] The mean age of seizure onset was calculated from a cohort of 268 individuals with GATOR1-related epilepsies reported in the literature.
[b] The distribution of epilepsy phenotypes was estimated from a collection of 152 GATOR1-related epilepsy pedigrees.
[c] Occipital lobe epilepsy, parietal lobe epilepsy or unspecified focal epilepsy.
[d] The frequency of cortical malformations in GATOR1-related epilepsies was estimated from a collection of 143 pedigrees. Reported malformations of cortical development included focal cortical dysplasia type I and II, bottom-of-sulcus dysplasia. hemimegalencephaly, subcortical heterotopia, polymicrogyria and pachygyria.
[e] 14 SUDEP cases in 155 *DEPDC5* pedigrees; 1 SUDEP case in 10 *NPRL2* pedigrees; 2 SUDEP cases in 18 *NPRL3* pedigrees[127].

A distinctive phenotype has been described with germline biallelic missense variants in *DEPDC5*[237]. This severe epilepsy syndrome was first described in 2022, in a series of nine children. Six children had Irish Traveller ancestry, two were of Tunisian ethnicity and one was of Lebanese origin. The Irish Traveller children had the same *DEPDC5* homozygous missense variant (p.Thr337Arg), while children with Lebanese and Tunisian ethnicity had a different *DEPDC5* homozygous missense variant (p.Arg806Cys). Shared phenotypic features included bilateral polymicrogyria, macrocephaly and early onset refractory epilepsy. Five of the children (55.55%) died in infancy or childhood[237].

Several studies suggest that pathogenic variants in GATOR1 genes confer a higher risk of SUDEP. In a series of 73 GATOR1-related epilepsy pedigrees, nine patients from eight families succumbed to definite or probable SUDEP[127]. Definite SUDEP was confirmed by autopsy in one case, while the remaining eight cases had probable SUDEP (without autopsy confirmation). The mean age at the time of SUDEP was 36.8 years[127]. A retrospective analysis of 61 SUDEP cases (92% autopsy confirmed) revealed that 10% of patients had pathogenic or likely pathogenic variants in *DEPDC5*[37]. Moreover, rodent models of *Depdc5* mTORopathy display a propensity for

terminal seizures, resembling the human phenomenon of SUDEP[143, 218, 260, 411]. Mice with CRISPR-Cas9-engineered focal mosaic *Depdc5* inactivation in brain exhibit clusters of focal-onset tonic-clonic seizures, followed by EEG suppression, and in some cases death[143].

Depdc5, *Nprl2* and *Nprl3* protein expression is elevated in mouse brain compared to other organs, with highest expression in brain cortex[130, 411]. GATOR1 proteins were expressed to a lesser degree in the medulla and heart. Cardiovascular defects were seen in *Depdc5* and *Nprl3* mutant mice who died *in utero*, supporting roles for these genes in cardiovascular development[412, 413]. Based on these preclinical observations, it was hypothesised that mutations in GATOR1 genes may cause primary cardiac alterations predisposing to SUDEP. Echocardiography, 12-lead electrocardiography (ECG), and holter monitoring found no clinical evidence of cardiac dysfunction in 16 patients with *DEPDC5* or *NPRL2/3* pathogenic variants, six of whom had a family history of SUDEP and three of whom subsequently died of SUDEP[411]. Post mortem examination of a female SUDEP patient with *DEPDC5*-related epilepsy found no evidence of structural cardiac pathology[411]. Simultaneous EEG-ECG monitoring of *Depdc5* deficient mice found no arrhythmias or dysautonomia before fatal seizures[411].

Drug-resistance and nocturnal seizures are well recognised risk factors for SUDEP, as well as being common features of GATORopathies. 'Pseudoresistance' may have contributed to SUDEP (autopsy-confirmed) in two brothers with *DEPDC5*-related epilepsy, as both were nonadherent with their ASMs[414]. It remains to be determined whether pathogenic variants in GATOR1 genes directly influence SUDEP risk, or if the increased prevalence of SUDEP merely reflects that GATOR1-related epilepsies commonly cause refractory focal epilepsies.

In a large cohort of individuals with epilepsy-related variants in GATOR1 genes, 96% were inherited and 4% occurred *de novo*[127]. LoF variants account for 60-70% of the GATOR1 mutational spectrum, consisting mostly of stop-gain (i.e., nonsense) and frameshift insertion or deletion (indel) variants (see **Table 6.1**)[127]. Stop-gain variants occur when a nucleotide substitution results in a premature stop codon (i.e., UAA, UAG, UGA). Frameshift variants (indel of a nucleotide sequence that is not divisible by three) can also produce premature stop codons.

Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that selectively degrades mRNAs harbouring premature stop codons. NMD was demonstrated in resected fresh-frozen brain[134], and cultured lymphoblasts[131-133] from patients with *DEPDC5* and *NPRL3* stop-gain or frameshift variants, indicating that haploinsufficiency is the pathogenic mechanism leading to LoF and resulting loss of inhibition of mTORC1. Haploinsufficiency describes the situation where having a single functioning copy of a gene is not enough for normal function, due to insufficient or absent production of the gene product. Recurrent LoF variants have been reported, raising the possibility of mutational hotspots or founder effects[127]. LoF variants in *DEPDC5* and *NPRL2*/*3* predict a more severe phenotype, with an increased frequency of FCD, epileptic spasms and SUDEP compared with missense variants[127].

Missense variants were reported in over 30% of probands with GATOR1-related epilepsies[127]. *In vitro* functional assessments of many GATOR1 missense variants have failed to demonstrate a deleterious effect on protein function[270, 271]. Moreover, strong evidence supporting their pathogenicity has not been obtained from segregation analysis or the presence of recurrent missense variants in unrelated probands. GATOR1 missense variants may lead to epilepsy phenotypes through distinct effects on GATOR1 function or mTORC1-independent mechanisms. However, some GATOR1 missense variants, previously predicted to have deleterious effects, may not impact the epilepsy phenotype, as they occur in genomic regions that are tolerant to variation. Baldassari and colleagues proposed an adapted classification framework for clinical interpretation of GATOR1 missense and splice-region variants, using gnomAD allele frequencies and *in silico* predictions of pathogenicity[127].

The introduction of this chapter provided an overview of the epidemiology, clinical features and genotype of GATOR1-related epilepsies as reported in the literature. Next, the clinical and genetic characteristics of a cohort of patients with GATOR1-related epilepsies attending Irish epilepsy clinics will be analysed. The objectives of this chapter are as follows:

a) To determine the frequency of GATOR1-related epilepsies attending the Beaumont Hospital epilepsy clinic.
b) To describe the clinical and genetic characteristics of an Irish cohort of patients with GATOR1-related epilepsies.

6.2 Methods

6.2.1 Estimated frequency of GATOR1-related epilepsies amongst patients attending the Beaumont Hospital epilepsy clinic

An epilepsy EPR search identified patients with epilepsy caused by single gene mutations (i.e., monogenic epilepsies) attending the Beaumont Hospital clinic. On the 3rd of March 2021, the EPR was searched for patients with "*definite*" OR "*probable*" genetic aetiology. Patients with IGE or epilepsy caused by chromosomal re-arrangements were excluded from the data search. Epilepsy-causative genetic variants had been detected by three different methods:

a) Research WES or WGS performed by the FutureNeuro research group, RCSI, Ireland.
b) Clinical epilepsy gene panels or WES performed by CeGat GmbH, Germany.
c) Patients who transitioned from paediatric neurology with an established genetic diagnosis.

Solved monogenic epilepsies diagnosed during the study period were added to the database following discussion at the epilepsy genetics MDT. The total number of epilepsy patients attending the clinic was estimated based on Delaney and colleagues' analysis of the Beaumont Hospital epilepsy cohort[76].

6.2.2 Clinical and genetic features of Irish patients with GATOR1-related epilepsies

Nine patients from seven families were studied. Six unrelated patients were identified via the genomic research programme at the FutureNeuro Research Centre. Three patients from the same family were identified through correspondence with paediatric neurology colleagues working at CHI at Temple Street. No additional cases were identified following correspondence with epilepsy specialists and paediatric neurologists at other Irish tertiary neurology centres.

All *DEPDC5*, *NPRL2* and *NPRL3* variants were initially detected by research WES, WGS or CNV analysis. All cases identified through the FutureNeuro genomic research programme were discussed at the epilepsy genetics MDT meeting with input from a

clinical geneticist, geneticist/bioinformaticians, neurologists and an epilepsy genetics research nurse[10]. Candidate variants were classified using ACMG guidelines[93]. Confirmation genetic testing was conducted by an accredited service provider (CeGat GmbH, Germany).

Patients with pathogenic or likely pathogenic variants in *DEPDC5* or *NPRL2/3* were included in the analysis. Patients with VUSs in GATOR1 genes were considered for inclusion by the epilepsy genetics MDT, if their phenotype was compatible with GATOR1-related epilepsy.

Demographic and clinical characteristics about Beaumont Hospital patients were retrieved from the epilepsy EPR, paper medical records and clinical interviews with patients, relatives and carers. Data collected included age, sex, seizure types, seizure frequency, age of seizure onset, MRI brain findings, EEG findings, current and prior ASMs, VNS, prior epilepsy surgery, and neuropsychiatric co-morbidities.

DRE was defined as '*failure of adequate trials of two tolerated and appropriately chosen and used ASM schedules (whether as monotherapies or in combination) to achieve sustained seizure contro*l'[61]. Seizure freedom was defined as "*freedom from seizures for a minimum of three times the longest preintervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever is longer*"[61]. Baseline seizure frequency was defined as the mean number of seizures per month, spanning the 3 months before the study period.

Genetic information about Beaumont Hospital patients was retrieved from the genomics module in the epilepsy EPR[198], CeGat genetic reports, and a database of sequencing results maintained by Dr. Katherine Benson. Clinical and genetic details on patients attending CHI at Temple Street were gathered from paper medical records and clinical interviews with the patients and their parents. Detailed pedigrees were constructed for all probands.

6.3 Results

6.3.1 Estimated frequency of GATOR1-related epilepsies amongst patients attending the Beaumont Hospital epilepsy clinic

Delaney *et al* estimated that 3,598 epilepsy patients were attending the Beaumont Hospital epilepsy clinic in 2018[76]. The EPR search identified 77 patients with solved monogenic epilepsies, representing 2.1% of the Beaumont Hospital epilepsy cohort (see **Figure 6.1**). TSC was the most common cause of monogenic epilepsy (22/77), followed by pathogenic variants in *SCN1A* (9/77). Four patients had pathogenic variants in *DEPDC5,* and two patients had pathogenic variants in *NPRL3*. Therefore, the estimated frequency of GATOR1-related epilepsies amongst patients with monogenic epilepsies was 7.8% (6/77), and 0.2% (6/3598) in the overall epilepsy cohort.

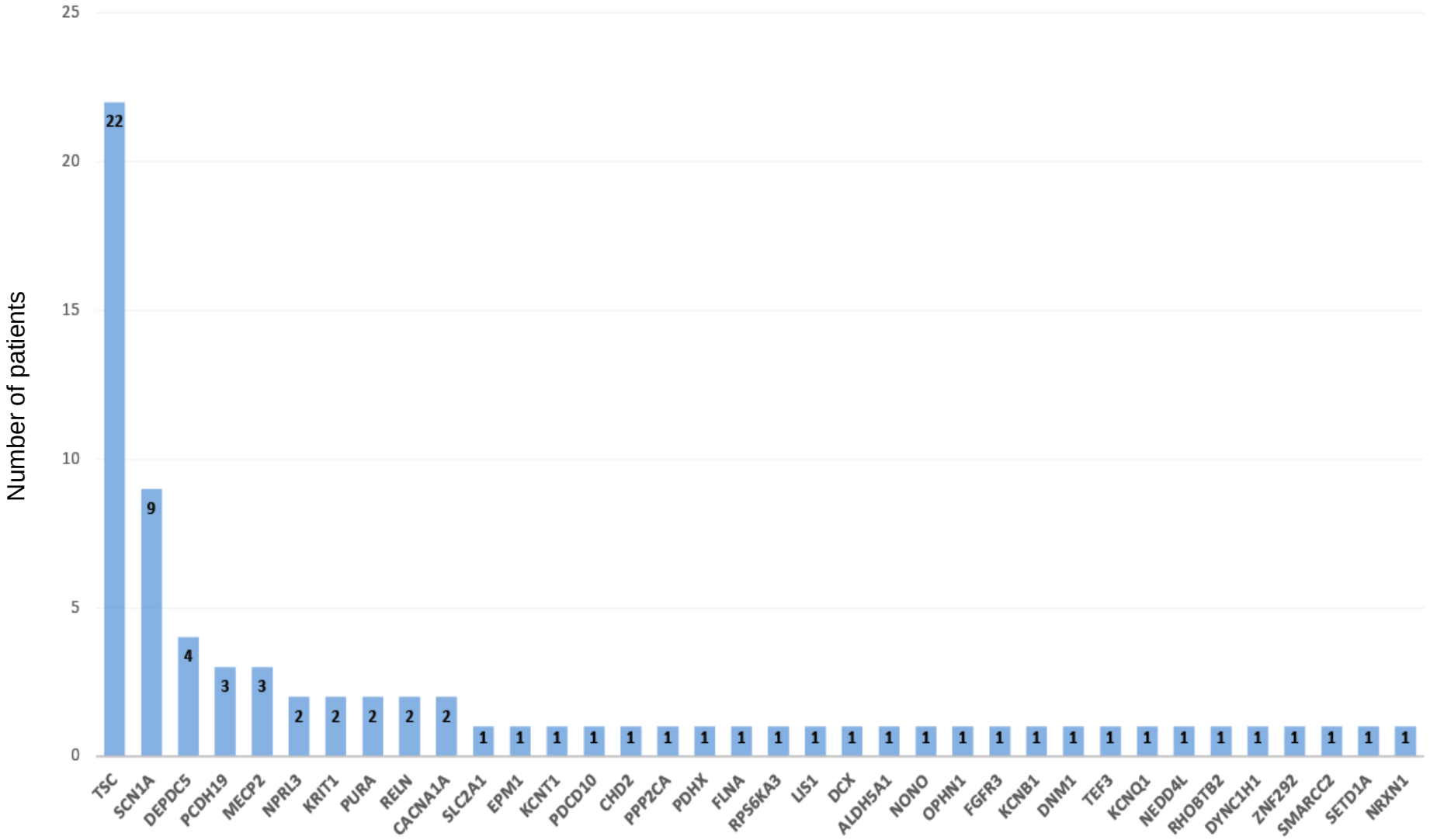

Figure 6.1 Solved monogenic epilepsies attending the Beaumont Hospital clinic

6.3.2 Clinical and genetic features of Irish patients with GATOR1-related epilepsies

Six unrelated patients (four males and two females) and three sisters (including two monozygotic twins) with GATOR1-related epilepsies were included in the analysis. Their main clinical features are presented in **Table 6.2**. The mean age was 36.8 years (range 13-63 years). Three of the six unrelated probands had a family history of epilepsy. Amongst unrelated patients, four had sleep-related hypermotor epilepsy and two had temporal lobe epilepsy. The three siblings had seizures emanating from different brain regions (frontal lobe epilepsy, parietal lobe epilepsy and temporal lobe epilepsy), consistent with FFEVF. The mean age of seizure onset was 8.1 years (range 4 weeks to 39 years). Two patients presented with seizures in the first year of life (22.2%), of whom one had infantile spasms (Patient 3 in **Table 6.2**).

The seizure spectrum comprised focal motor seizures with maintained awareness (1/9, 11.1%), focal motor seizures (hyperkinetic) with impaired awareness (4/9, 44.44%), focal non-motor seizures with impaired awareness (4/9, 44.44%) and focal to bilateral tonic-clonic seizures (5/9, 55.55%), although only two patients had convulsions in the previous year. One patient had a remote history of focal motor SE. Seizures occurred predominantly from sleep in 44.44% (4/9) of patients. All patients had EEG investigations, of whom five had prolonged vEEG monitoring. No patients underwent invasive EEG monitoring. Six patients (66.66%) had interictal epileptiform discharges (three unifocal and three multifocal). One patient had interictal focal slowing, while two had normal interictal EEG. In the five patients who underwent vEEG monitoring, two had regional paroxysmal fast activity at the onset of seizures (40%), two had focal rhythmic sharp wave activity at the onset of seizures (40%), and one patient had normal surface EEG recordings during seizures (20%). One patient had post-ictal asystole (8 seconds) on telemetry ECG, prompting cardiac pacemaker insertion (Patient 2 in **Table 6.2**). All patients had normal MRI brain studies (three had 3-Tesla MRI and six had 1.5-Tesla MRI). One patient had focal hypometabolism on FDG-PET brain imaging, which corresponded with the ictal onset zone on EEG (Patient 1 in **Table 6.2**).

Table 6.2 Clinical features of Irish patients with GATOR1-related epilepsies

n	Gender	Age (yrs)	Age of onset (yrs)	Epilepsy phenotype	Seizure types	Seizure frequency	ASMs	Number of prior ASMs	VNS or epilepsy surgery	MRI brain	EEG	Cognitive and psychiatric features	GATOR1 gene and inheritance
1	Male	49	4	Sleep-related hypermotor epilepsy	FIA from sleep *(hyperkinetic)*	86 per month	ESL LTG PHT VLP	4	No	Normal (1.5-T)	Right parietal interictal sharp waves, right hemisphere paroxysmal fast activity at onset of seizure	Psychotic depression on long-term olanzapine treatment	*DEPDC5* Mother had nocturnal epilepsy *(deceased)*
2	Male	46	8	Sleep-related hypermotor epilepsy	FIA from sleep *(hyperkinetic)*	11 per month	BRV PER CBZ	13	No	Normal (3-T)	Right frontal interictal sharp waves, right temporal paroxysmal fast activity at onset of seizures, post-ictal asystole on ECG	No	*DEPDC5* 3 sisters, 1 niece and 1 maternal cousin had epilepsy *(all declined genetic testing)*
3	Female	33	0.1	Sleep-related hypermotor epilepsy	FIA from sleep *(hyperkinetic)* Remote history of FBTC and infantile spasms	49 per month	CBZ PER LCM	14	VNS	Normal (1.5-T)	Slow background, right parietal and left temporal interictal sharp waves and right parasagittal ictal onsets	Mild ID, anxiety, psychogenic non-epileptic seizures	*NPRL3* No family history of epilepsy *(parents not tested)*
4	Male	35	3	Temporal lobe epilepsy	FIA *(emotional, cognitive)* FBTC	18 per month	CBZ LCM	16	VNS	Normal (3-T)	Slow background, bitemporal interictal discharges and left temporal ictal onsets	Mild ID, anxiety, depression, psychosis, psychogenic non-epileptic seizures	*DEPDC5* No family history of epilepsy *(parents not tested)*

5	Male	62	0.75	Sleep-related hypermotor epilepsy	FIA from sleep *(hyperkinetic)*	Seizure-free	LEV TPM LTG	8	VNS	Normal (1.5-T)	No interictal abnormalities and no surface EEG changes at onset of seizures	No	*NPRL3* Father had nocturnal epilepsy (*deceased*)
6	Female	63	39	Temporal lobe epilepsy	FIA *(automatism)* FBTC	Seizure-free	CBZ VLP	0	No	Normal (1.5-T)	Left temporal interictal slowing	No	*DEPDC5* Son has tumour-related epilepsy *(DEPDC5 variant negative)*
7	Female	15	2	Frontal lobe epilepsy (FFEVF)	FIA *(automatism)* FBTC	8 per month	LCM ESL PER	5	No	Normal (3-T)	Bilateral mesial frontal interictal sharp wave discharges	Dyslexia, anxiety disorder	*DEPDC5* Familial variant inherited from mother
8	Female	15	4	Parietal lobe epilepsy (FFEVF)	FA *(motor)* Remote history of focal SE	1 per year	OXC	7	No	Normal (1.5-T)	Left parietal interictal sharp waves	No	*DEPDC5* Familial variant inherited from mother
9	Female	13	9	Temporal lobe epilepsy (FFEVF)	FIA *(automatism)* FBTC	Seizure-free	OXC LEV CLB	0	No	Normal (1.5-T)	Normal	Mild ID	*DEPDC5* Familial variant inherited from mother

Abbreviations:
ASM= anti-seizure medication; BRV= brivaracetam; CBZ= carbamazepine; CLB= clobazam; ECG= electrocardiogram; EEG= electroencephalography; ESL= eslicarbazepine; FA= focal aware; FBTC= focal to bilateral tonic-clonic; FFEVF= familial focal epilepsy with variable foci; FIA= focal impaired awareness; ID= intellectual disability; LCM= lacosamide; LEV= levetiracetam; LTG= lamotrigine; MRI= magnetic resonance imaging; OXC= oxcarbazepine; PER= perampanel; PHT= phenytoin; SE= status epilepticus; T= tesla; TPM= topiramate; VLP= valproic acid; VNS= vagus nerve stimulation.

Three patients were seizure-free, and one patient had infrequent focal motor seizures with maintained awareness (approximately one per year). The remaining five patients had active DRE (55.55%), with a mean seizure frequency of 34.4 per month (range 8-86 per month). The median number of current ASMs was three (range 1-4 ASMs). The median number of previously failed ASMs was seven (range 0-16 ASMs). Three patients had VNS. No patients had prior epilepsy surgery. Among seizure-free patients, one patient achieved seizure freedom on VNS therapy after a long history of active DRE (Patient 5 in **Table 6.2**), one patient achieved seizure freedom on carbamazepine and valproate combination therapy (Patient 6 in **Table 6.2**), and another patient on oxcarbazepine, levetiracetam and clobazam (Patient 9 in **Table 6.2**). The patient with infrequent focal sensory seizures is maintained on oxcarbazepine monotherapy (Patient 8 in **Table 6.2**). No patients succumbed to SUDEP. The mother of Patient 1 died in her sleep at the age of 54 years. It was suspected that the cause of death may have been cardiac in nature, but SUDEP was also a potential cause, as she had a lifelong history of nocturnal epilepsy.

Cognitive deficits and/or psychiatric co-morbidities were present in 55.55% (5/9) of patients. Two patients had mild ID, one patient had dyslexia and dyscalculia, and one patient was awaiting formal neuropsychology assessment. Four patients had significant psychiatric disorders, including depression, anxiety disorder and psychosis. Two patients (22.22%) with active DRE had PNES diagnosed by vEEG (Patients 3 and 4 in **Table 6.2**).

Amongst unrelated probands, four had *DEPDC5* variants and two had *NPRL3* variants (**Table 6.3**). The three siblings had deletion of exons 12-17 in the *DEPDC5* gene. All variants in GATOR1 genes were classified as pathogenic or likely pathogenic by ACMG criteria, apart from the *DEPDC5* missense variant in Patient 4, which was classified as a VUS. The clinical significance of the *DEPDC5* missense variant was discussed at the epilepsy genetics MDT. We decided to include Patient 4 based on the compatibility of his phenotype with GATOR1-related epilepsy, and the variant's very low frequency in population databases (gnomAD allele count of 1). Two patients had stop-gain *DEPDC5* variants, and two patients had frameshift variants (microdeletion in *DEPDC5* and microinsertion in *NPRL3).* Stop-gain and frameshift variants in GATOR1 genes are predicted to cause LoF through haploinsufficiency.

Intragenic deletions in *DEPDC5,* such as the deletion of exons 12-17 described here, have been previously documented in patients with focal epilepsy and are thought to result in LoF[127]. A summary of each patient's phenotype and pedigree is outlined below.

Table 6.3 Genetic characteristics in patients with GATOR1-related epilepsies

n	Gene	Variant	Variant type	GnomAD allele count	ACMG category	ACMG Evidence
1	*DEPDC5*	NM_001242896.3 c.3436C>T, (p.Gln1146Ter)	Stop-gain	0	Pathogenic	PVS1[a], PM2[b], PP5[c]
2	*DEPDC5*	NM_001242896.3 c.675delC, (p.Tyr226fs)	Frameshift deletion	0	Likely pathogenic	PVS1[a], PM2[b]
3	*NPRL3*	NM_001077350.3 c.189-1G>A	Splicing	0	Pathogenic	PVS1[a], PM2[b], PP5[c]
4	*DEPDC5*	NM_001242896.3 c.4283G>A, (p.Ser1428Asn)	Missense	1	VUS	BP4[d]
5	*NPRL3*	NM_001077350.3 c.653_654insCCCG, (p.Leu219fs)	Frameshift insertion	0	Likely pathogenic	PVS1[a], PM2[b]
6	*DEPDC5*	NM_001242896.3 c.2512C>T, (p.Arg838Ter)	Stop-gain	0	Pathogenic	PVS1[a], PM2[b], PP5[c]
7-9	*DEPDC5*	Chr22: g.31796907_31805710del	Deletion	0	Pathogenic	PVS1[a], PM1[e], PM2[b]

Footnotes:
[a] PVS1 is defined as "null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss-of-function is a known mechanism of disease"[93].
[b] PM2 is defined as absent from controls in Exome Sequencing Project, 1000 Genomes or ExAC[93].
[c] PP5 is defined as "reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation"[93].
[d] BP4 is defined as "multiple lines of computational evidence suggest no impact on gene or gene product"[93].
[e] PM1 is defined as "located in a mutational hot spot and/or critical and well-established functional domain without benign variation"[93].

Abbreviations:
ACMG= American College of Medical Genetics and Genomics; VUS= variant of uncertain significance.

6.3.3 Patient 1: NM_001242896.3(DEPDC5): c.3436C>T (p.Gln1146Ter)

This 49-year-old man had seizures since the age of four years. Throughout childhood and early adulthood, he had infrequent seizures (1-2 per year) on phenytoin monotherapy. Seizure control deteriorated in his 40's following an attempt to gradually substitute levetiracetam for phenytoin to reduce his risk of long-term complications. Over the next five years, his seizure frequency increased to at least two focal hyperkinetic seizures with impaired awareness every night. His ASMs were changed several times, including reinstating phenytoin. His current ASMs comprised phenytoin 225mg/day, eslicarbazepine 800mg/day, lamotrigine 250mg/day and valproate prolonged release 1200mg/day. He did not respond to levetiracetam, clobazam, perampanel, zonisamide and pregabalin. vEEG monitoring confirmed a right frontoparietal epilepsy focus. Brain MRI was normal. FDG-PET brain revealed focal hypometabolism in the right parietal region. He was deemed to be a potential epilepsy surgery candidate but declined invasive EEG monitoring. His neuropsychiatric history was significant for severe psychotic depression aged 44 years, requiring hospitalisation and long-term treatment with olanzapine. His deceased mother also had nocturnal seizures (see **Figure 6.2** for pedigree). Research WES detected the *DEPDC5* stop-gain variant in 2019, classified as pathogenic by ACMG criteria.

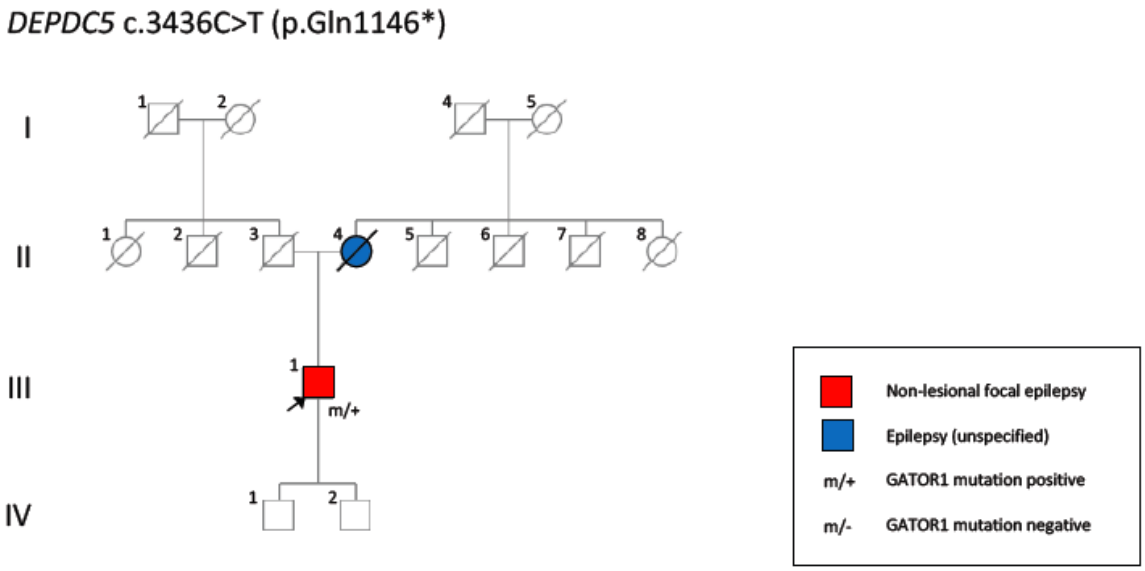

Figure 6.2: Pedigree for Patient 1

6.3.4 Patient 2: NM_001242896.3(DEPDC5):c.675del (p.Tyr226fs)

This 46-year-old man had sleep-related hypermotor epilepsy since the age of eight years. Throughout adulthood, he had two to three nocturnal focal hyperkinetic seizures with impaired awareness per week. He was treated with brivaracetam 275mg/day, carbamazepine prolonged release 1000mg/day and perampanel 6mg/day. He had failed 13 prior ASMs. The patient reported experiencing a period of good seizure control while taking valproate in his 30's. Valproate was stopped due to concerns that it was contributing to male infertility. Prior vEEG confirmed right frontal ictal onsets. A cardiac pacemaker was inserted for post-ictal asystole, detected during vEEG monitoring. 3-Tesla Brain MRI was normal. He declined invasive EEG monitoring to determine his suitability for epilepsy surgery. Research WES identified the *DEPDC5* frameshift microdeletion in 2020, classified as likely pathogenic by ACMG criteria. He has three sisters, one niece and a maternal first cousin with well-controlled epilepsy, all of whom declined genetic testing (see **Figure 6.3** for pedigree).

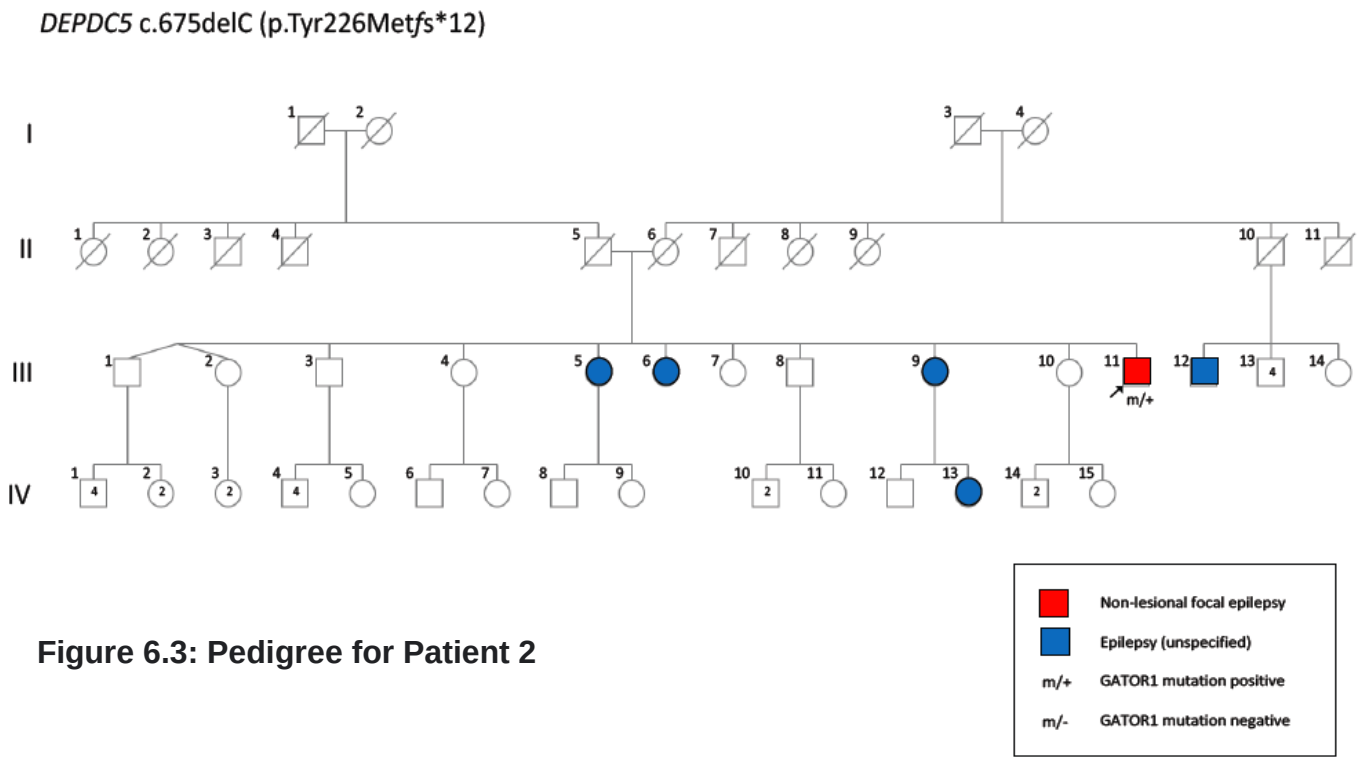

Figure 6.3: Pedigree for Patient 2

6.3.5 Patient 3: NM_001077350.3(NPRL3):c.189-1G>A

This 33-year-old woman began having seizures in the first month of life. Infantile spasms emerged before the age of six months and responded to adrenocorticotropic hormone (ACTH) treatment. Throughout childhood and early adulthood, nocturnal focal hyperkinetic seizures were the predominant seizure type, with rare focal to bilateral tonic-clonic seizures. At baseline, she had at least one hyperkinetic seizure with impaired awareness per night. She was taking carbamazepine prolonged release 1000mg/day, lacosamide 100mg/day and perampanel 4mg/day. She had failed 14 prior ASMs and VNS. Prior vEEG showed excessive bihemispheric theta and delta slowing, multifocal interictal epileptiform discharges (right parietal and left temporal) and seizures arising from the right parasagittal region, consistent with multifocal epilepsy. Brain MRI was normal. She had mild ID and resided in a supervised independent living setting. She had significant psychiatric co-morbidities including anxiety disorder and PNES. She had no relatives with epilepsy or ID (see **Figure 6.4** for pedigree). Research WES detected the *NPRL3* splice-site variant in 2020, classified as pathogenic by ACMG criteria.

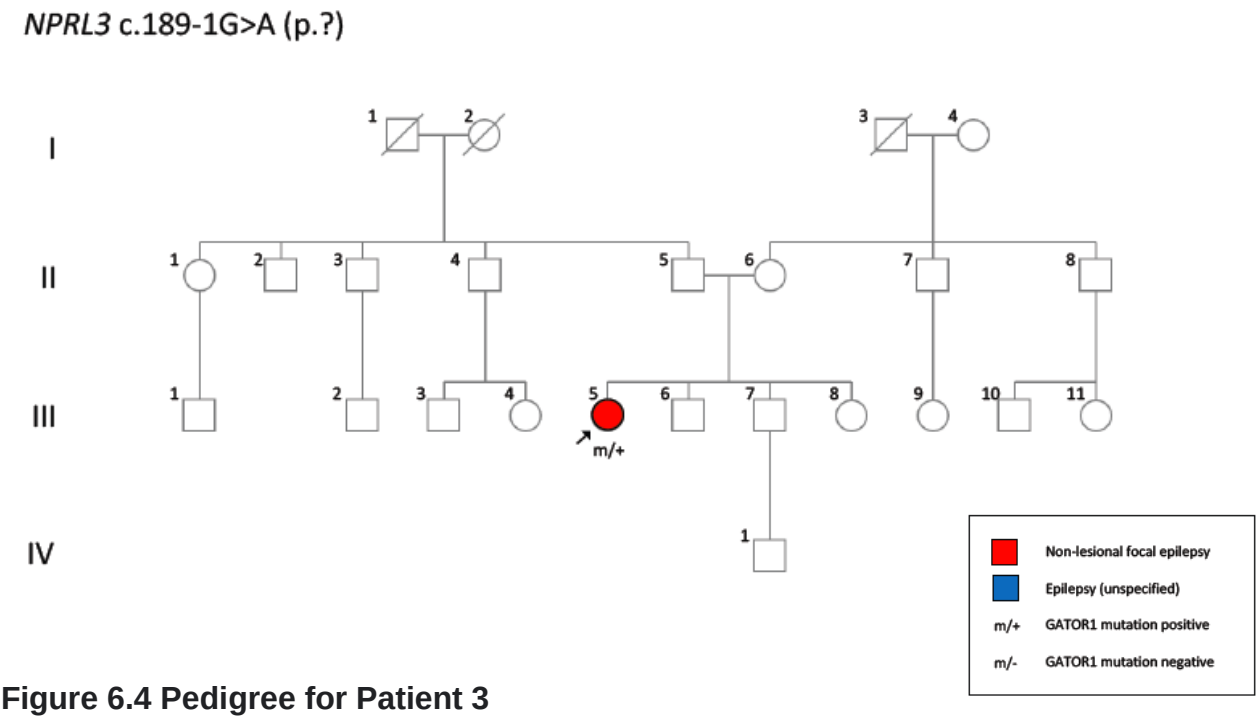

Figure 6.4 Pedigree for Patient 3

6.3.6 Patient 4: NM_001242896.3(DEPDC5):c.4283G>A (p.Ser1428Asn)

This 35-year-old man had seizures since the age of three years. As a child, he had infrequent focal seizures with temporal lobe semiology. Seizure control deteriorated in his teenage years with prominent peri-ictal psychiatric symptoms. Throughout adulthood, he had two to three focal impaired awareness seizures with emotional and cognitive features per week. In addition, he had at least three focal to bilateral tonic-clonic seizures per month. He was treated with lacosamide 400mg/day and carbamazepine prolonged release 1200mg/day, having failed 16 prior ASMs and VNS. Prolonged vEEG monitoring showed very frequent slowing (theta and polymorphic delta activity), independent epileptiform discharges over both temporal regions, and seizures arising from the left temporal region, consistent with multifocal epilepsy. Brain MRI was normal. He had mild ID and prominent verbal dysfluency. He resided in a supervised independent living setting with carer support. He had severe psychiatric symptoms, including depression, anxiety and psychosis. In addition, PNES were diagnosed during a vEEG admission. He had a paternal first cousin once removed with epilepsy and autism (IV-1 in pedigree, **Figure 6.5**). He wasn't available for genetic testing. A *DEPDC5* missense variant was detected by research WES in 2019, classified as a VUS by ACMG criteria.

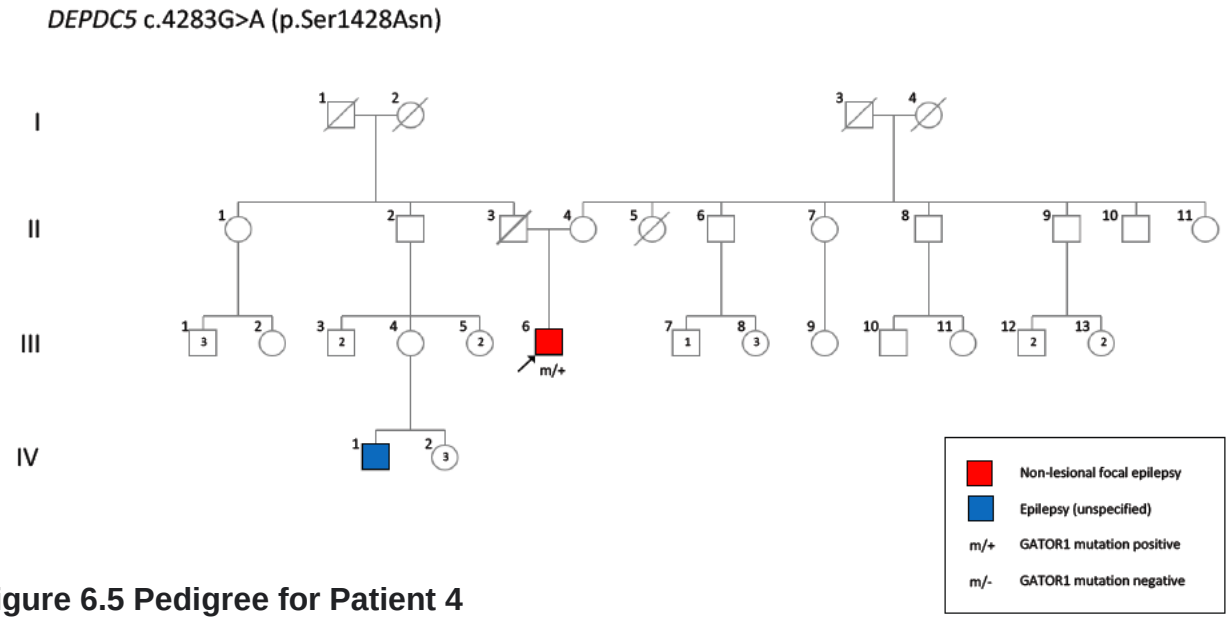

Figure 6.5 Pedigree for Patient 4

6.3.7 Patient 5: NM_001077350.3(NPRL3): c.653_654insCCCG (p.Leu219fs)

This 62-year-old man had sleep-related hypermotor epilepsy since the age of nine months. He had intractable nocturnal seizures with hyperkinetic limb movements and back-arching until his mid 40's. He was treated with levetiracetam 2500mg/day, topiramate 100mg/bd and lamotrigine 400mg/day, having not responded to carbamazepine, valproate, gabapentin, oxcarbazepine, phenytoin, primidone, vigabatrin and clobazam. He experienced a remarkable reduction in seizures following VNS insertion aged 41 years. Over the subsequent 20 years, he had infrequent focal seizures, usually in the context of intercurrent infection. He was seizure-free for four years. Previous vEEG showed seizure semiology consistent with sleep-related hypermotor epilepsy, with no interictal epileptiform discharges or accompanying ictal EEG activity. MRI brain was normal. His deceased father had nocturnal epilepsy (see **Figure 6.6** for pedigree). Research WES detected the *NPRL3* frameshift microinsertion in 2020, classified as likely pathogenic by ACMG criteria.

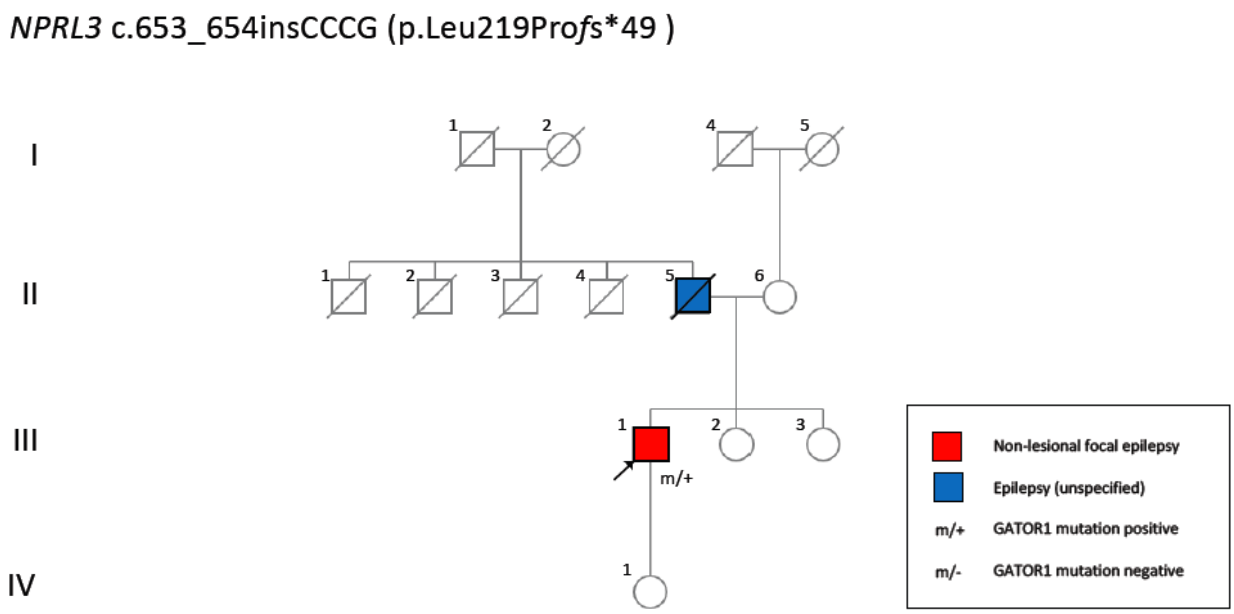

Figure 6.6 Pedigree for Patient 5

6.3.8 Patient 6: NM_001242896.3(DEPDC5):c.2512C>T (p.Arg838Ter)

This 63-year-old woman began having seizures during pregnancy aged 42 years. Her seizures comprised vacant staring, oral and manual automatisms, and loss of awareness. In total, she had approximately 10 focal to bilateral tonic-clonic seizures from sleep. She was seizure-free for more than 20 years on valproate 2000mg/day and carbamazepine prolonged release 1200mg/day. No other ASMs were trialled. EEG showed interictal left temporal slowing. MRI brain was normal. She had no neuropsychiatric co-morbidities. The *DEPDC5* stop-gain variant was detected by research WES in 2020, classified as pathogenic by ACMG criteria. This variant was already reported in patients with sleep-related hypermotor epilepsy and frontal lobe epilepsy[127]. Her oldest son has focal DRE in the context of a surgically resected oligodendroglioma in childhood. Cascade testing did not identify the *DEPDC5* variant in her son with epilepsy (see **Figure 6.7** for pedigree).

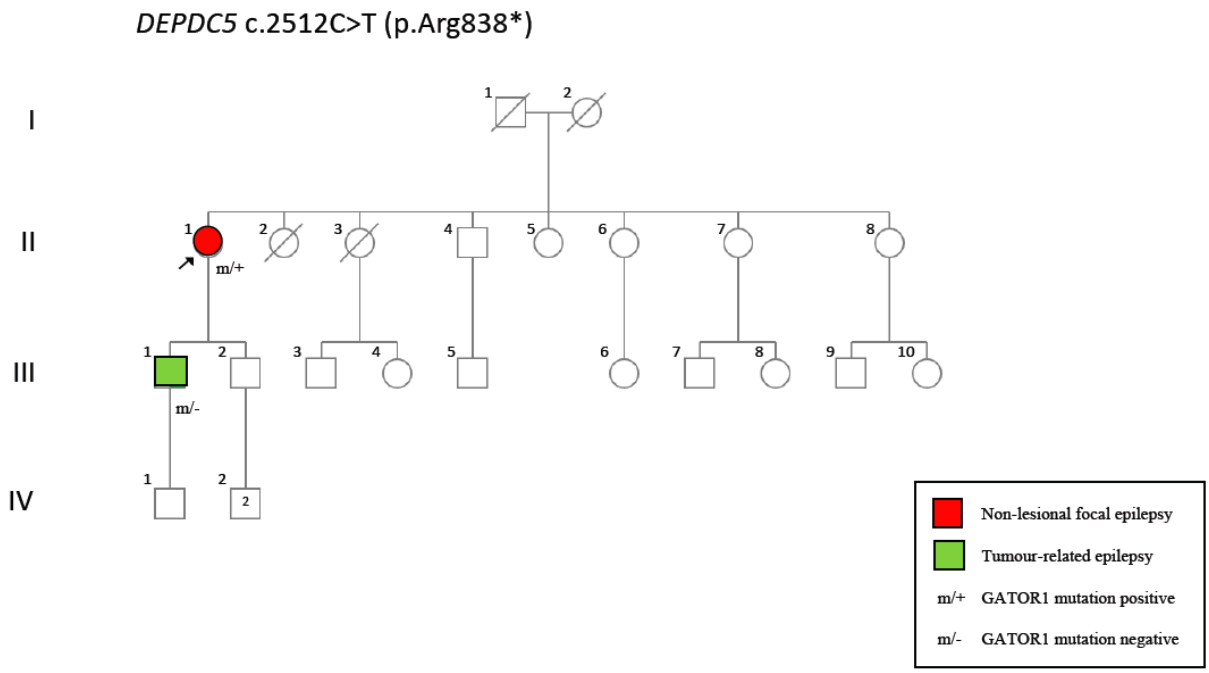

Figure 6.7 Pedigree for Patient 6

6.3.9 Patients 7-9: *DEPDC5* Chr22: g.31796907_31805710del

The proband (IV-2 in **Figure 6.8**) was a 15-years-old female with focal epilepsy since the age of two years. Her seizures were stereotyped, with facial grimacing, oral and manual automatisms, followed by loss of awareness. At baseline, she had eight focal impaired awareness per month. These occasionally progressed to bilateral tonic-clonic seizures. Her seizures clustered around her menstrual period. She was treated with lacosamide 600mg/day, eslicarbazepine 1200mg/day and perampanel 5mg/day. She had failed five prior ASMs. Overnight EEG displayed bilateral mesial frontal interictal epileptiform discharges, but no seizures were recorded. 3-Tesla MRI brain was normal. She had normal early life development and achieved age-appropriate motor and cognitive milestones. During primary school, she struggled academically and a formal educational psychology assessment diagnosed dyslexia, dyscalculia and dyspraxia. Neuropsychiatric symptoms were prominent, including low mood, anxiety and angry outbursts. The familial *DEPDC5* variant was detected by research CNV analysis. The variant was inherited from her asymptomatic mother (III-2 in **Figure 6.8**). Her maternal aunt (III-3) has inactive non-lesional focal epilepsy, but tested negative for the familial *DEPDC5* variant.

The proband's identical twin (IV-3 in **Figure 6.8**) developed seizures aged four years. Her seizures comprise a sequence of a rising epigastric sensation, right leg pain and then jerking of her right leg, without loss of awareness. Aged four years, she had a long hospital admission with epilepsia partialis continua involving her right leg. After trialling several ASMs, she achieved seizure control on oxcarbazepine, levetiracetam and clobazam. Clobazam and levetiracetam were successfully weaned, and she was maintained on oxcarbazepine monotherapy (2340mg/day). She had rare breakthrough seizures if her ASM was missed. Routine EEG revealed left mesial parietal interictal epileptiform discharges, consistent with her seizure semiology. Brain MRI was normal. She had no neuropsychiatric co-morbidities or difficulties at school.

The proband's younger sister (IV-1 in **Figure 6.8**) was 13-years-old. Her seizures began aged nine years. She had focal seizures with automatisms and altered awareness, consistent with temporal lobe epilepsy. She was seizure-free for 18 months on oxcarbazepine 1200mg/day, levetiracetam 600mg/day and clobazam

10mg/day. Routine EEG and MRI brain were normal. She had academic difficulties at school and was awaiting formal psychology assessment.

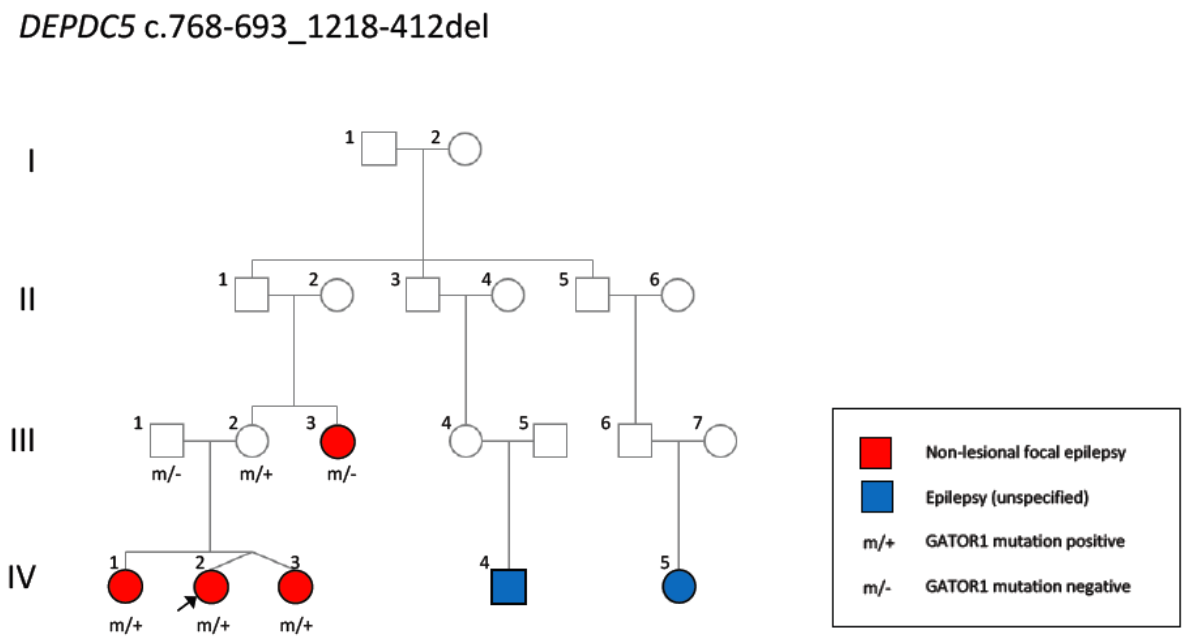

Figure 6.8 Pedigree for Patients 7-9

6.4 Discussion

In this chapter, I investigated the frequency of GATOR1-related epilepsies in patients attending the Beaumont Hospital epilepsy clinic, and deeply phenotyped a cohort of Irish patients with epilepsy-causative *DEPDC5* and *NPRL3* variants. GATOR1-related epilepsies were the third most common cause of monogenic epilepsy in patients attending the clinic, after TSC and *SCN1A*-related epilepsies. This finding aligns with previous work that identified pathogenic variants in GATOR1 genes as a common cause of familial focal epilepsy[130, 131, 402]. However, GATOR1-related epilepsies made up less than 0.2% of the overall Beaumont Hospital cohort, indicating that sporadic epilepsies are rarely caused by *DEPDC5* or *NPRL2*/3 mutations[403]. Coordinated efforts to sequence patients with refractory non-lesional focal epilepsies will likely increase the diagnostic rate of GATOR1-related epilepsies, as previous genomic testing initiatives in the field of epilepsy have largely focused on DEE.

The phenotypes of nine patients with GATOR1-related epilepsies were analysed, including three sisters with FFEVF. The cohort exhibited the full spectrum of focal-onset seizures, with variable interictal and ictal EEG findings. All had difficult-to-treat focal epilepsies. Five patients had ongoing active DRE. The four patients with good seizure control required ASM combination therapy or VNS to achieve seizure remission. Similarly, in the largest cohort study of GATOR1-related epilepsies approximately 60% of patients had DRE[127].

No Irish patients had FCD detected on MRI brain. Baldassari *et al* found that one-fifth of patients with GATOR1-related epilepsies had FCD[127]. Among patients with GATOR1-related epilepsies who underwent epilepsy surgery, most had neuropathological findings of FCD type II, including those with normal MRI brain[127]. It is possible that some patients in the present study had FCD that was not detectable by clinical MRI. Patients 1 and 2 had regional paroxysmal fast activity at the onset of seizures on scalp EEG. This ictal EEG pattern, which is associated with FCD type II[415], might indicate the presence of FCD in these two patients. Epilepsy surgery was considered in Patients 1 and 2 but both declined invasive EEG monitoring to better localise ictal-onset zones. Epilepsy surgery for refractory GATOR1-related epilepsies

resulted in favourable seizure outcomes in 80% of patients in Baldassari *et al's* cohort study[127].

The pedigree of Patients 7-9 illustrates several important clinical features of GATOR1-related epilepsies, including intrafamilial variability and incomplete penetrance. The three sisters had epilepsies originating from different brain regions, with variable age of onset and responsiveness to ASMs. These phenotypic differences were particularly remarkable in the monozygotic twins with identical genotypes. These differences may reflect epigenetic factors, environmental influences, or the presence of "second-hit" somatic mutations, which have been reported in *DEPDC5*-related epilepsies[90, 91, 143, 234]. The sisters inherited the *DEPDC5* variant from their asymptomatic mother, consistent with incomplete penetrance. Similarly, the pedigree of Patient 2 suggests incomplete penetrance. The proband had three affected siblings and an affected maternal first cousin, but his deceased mother was asymptomatic (see **Figure 6.3**).

Risk factors for SUDEP were common amongst Irish patients with GATOR1-related epilepsies, including nocturnal seizures, DRE and polypharmacy[26, 27]. Patient 1 had a family history suggestive of SUDEP. Of interest, Patient 2 had a cardiac pacemaker implanted for ictal asystole detected during vEEG monitoring. Ictal asystole is a potential mechanism underlying SUDEP in all epilepsies, including the GATOR1-related epilepsies. Bacq and colleagues found no evidence of structural or electrical cardiac abnormalities in 16 patients with GATOR1-related epilepsies, of whom six had a family history of SUDEP and three ultimately died of SUDEP[411]. The study was limited by its small sample size, so further research is needed to fully understand the role of ictal bradyarrhythmia and asystole in SUDEP risk in GATOR1-related epilepsies.

This deep phenotyping analysis demonstrated the significant neuropsychiatric co-morbidities observed in GATOR1-related epilepsies. Five out of nine patients (55.55%) had psychiatric co-morbidities, including depression, anxiety and psychosis. Only two of six adults with GATOR1-related epilepsies were able to maintain long-term employment, highlighting the hidden disability often associated with monogenic epilepsies. Two patients (Patients 3 and 4) with refractory GATOR1-related epilepsies and co-morbid ID had PNES and epileptic seizures recorded during the same period

of vEEG monitoring. To the best of my knowledge, coexisting epilepsy and PNES in patients with *DEPDC5* or *NPRL3* mutations has not been previously described in the literature. We previously reported that 7.3% of patients (19/262) monitored by vEEG in Beaumont Hospital between 2013 and 2015 had epileptic seizures and PNES recorded during the same admission[55]. Co-morbid psychiatric disorders (37.5%) and ID (25%) were common amongst patients with a dual diagnosis of epilepsy and PNES. Underlying cognitive and psychiatric impairments likely contributed to the emergence of PNES in these two patients. Leu *et al* found that a subset of patients with PNES without coexisting epilepsy had deleterious variants in genes associated with monogenic neurological and psychiatric disorders, suggesting that genetic factors may be involved in the development of PNES[416].

The main weakness of this study was the absence of functional characterisation of GATOR1 genetic variants. LoF was inferred with stop-gain, frameshift and deletion variants, and the *NPRL3* variant's deleterious effect on splicing was predicted based on its location in an essential splice-site. Functional assessments of many GATOR1 missense variants have shown no evidence of mTORC1 hyperactivation, making assignment of pathogenicity difficult[271]. A novel mTORC1 functional assay was successfully used to resolve *SZT2* VUSs[272], and potentially could be utilised to characterise GATOR1 missense variants, such as the *DEPDC5* missense VUS present in Patient 4.

In summary, GATOR1-related epilepsies are often difficult to treat and carry a disproportionate risk of SUDEP. Dysregulated GATOR1 inhibition leading to excessive mTORC1 activation appears to be involved in the development of epilepsy in GATORopathies. Preclinical models[262, 263, 271] and resected brain tissue from patients with GATOR1-related epilepsies[90, 131, 134] have shown evidence of mTORC1 hyperactivation. As mTOR inhibitors have proven to be efficacious and safe treatments for DRE in TSC and PMSE, they represent a promising therapeutic strategy in GATOR1-related epilepsies[135, 215]. In the next chapter, the potential of everolimus as a precision treatment for refractory GATOR1-related epilepsies is explored.

7. Everolimus as a precision therapy for the GATOR1-related epilepsies: a pilot observational study

7.1 Introduction

In the previous chapter, I described the clinical and genetic characteristics of a cohort of patients with GATOR1-related epilepsies. Five out of nine (55.55%) patients had active DRE, with a mean seizure frequency of 34 per month over the previous three months. This study expands on the work of Baldassari *et al,* who found that 54% (38/71) of probands with pathogenic variants in GATOR1 genes had DRE[127]. Several studies also suggest that pathogenic variants in GATOR1 genes confer a higher risk of SUDEP[37, 127, 411, 414].

The GATOR1 complex inhibits mTORC1 activity in response to intracellular amino-acid levels. Nonsense-mediated mRNA decay (NMD) was demonstrated in lymphoblast cell lines and resected brain tissue from patients with truncating variants in *DEPDC5* and *NPRL3*, confirming haploinsufficiency as the mechanism of pathogenicity[131-134]. *DEPDC5* and *NPRL3* haploinsufficiency results in mTORC1 hyperactivation, as demonstrated in rodent models[263, 417], and human brain specimens[131, 134, 278]. As previously discussed, many rare GATOR1 missense variants lack supporting evidence for pathogenicity from *in vitro* functional testing and familial segregation[127, 271].

Analogous to TSC, excessive mTORC1 activation appears to be a key driver of seizures in GATOR1-related epilepsies. The demonstration of rapamycin-responsive seizures in mouse models of *Depdc5, Nprl2* and *Nprl3* knockout[262, 263], coupled with the clinical success of everolimus treatment in TSC-related epilepsy has given rise to the hypothesis that mTOR inhibitors may ameliorate seizures in GATOR1-related epilepsies[136].

To begin to test this hypothesis, an open-label pilot observational study of adjunctive everolimus for DRE caused by variants in *DEPDC5* or *NPRL2*/*3* was conducted.

7.2 Methods

7.2.1 Participants

A total of five patients participated in this study. Three adult patients were recruited from Beaumont Hospital and one adult patient from Cork University Hospital. The four adult patients were identified via the genomic research programme at the FutureNeuro Research Centre. All cases identified through FutureNeuro genomic research were discussed at the epilepsy genetics MDT meeting. One additional case was identified through correspondence with paediatric neurology colleagues working in CHI at Temple Street.

DEPDC5, NPRL2 and *NPRL3* variants were detected by research WES, WGS or CNV analysis. Candidate variants were classified using ACMG guidelines[93]. Confirmation genetic testing was conducted by an accredited service provider (CeGat GmbH, Germany).

DRE patients aged two years or older with pathogenic or likely pathogenic variants in *DEPDC5* or *NPRL2*/*3* were included in the study. DRE was defined as '*failure of adequate trials of two tolerated and appropriately chosen and used ASM schedules (whether as monotherapies or in combination) to achieve sustained seizure control'* [61]. Patients with VUSs in GATOR1 genes were considered by the epilepsy genetics MDT for possible inclusion provided their phenotype was compatible with GATOR1-related focal epilepsy.

7.2.2 Study Procedure

Everolimus was initiated at 8mg/m^2 daily in patients taking concomitant CYP3A4 inducers and 5mg/m^2 daily in patients not taking CYP3A4 inducers[308]. The everolimus dose was titrated to a target blood trough concentration of 5-15ng/mL. Baseline seizure frequency was defined as the mean number of seizures per month, spanning the 3 months prior to everolimus initiation. All ASM doses and VNS settings were stable for at least one month prior to starting everolimus. Patients required continuous follow-up for at least six months after everolimus initiation. Additional ASMs were not introduced during the study period.

The primary outcome measure was change in mean monthly seizure frequency (MMSF) compared with baseline seizure frequency recorded on seizure diary. The MMSF was calculated by dividing the total number of seizures on treatment by the number of months on treatment. Treatment responders were defined as those with a greater than 50% MMSF reduction from baseline seizure frequency at last review on everolimus. Patient or caregiver rating on the Clinical Global Impression of Improvement (CGI-I) scale was recorded as a secondary outcome measure (see **Appendix 6**). Treatment-emergent adverse events were recorded using a structured questionnaire utilised in a study of everolimus for infants with TSC and graded according to the Common Terminology Criteria for adverse events (see **Appendix 5**)[340]. Adjustments to concomitant ASMs were also recorded.

7.2.3 Ethical approval and patient consent

This study was approved by the Beaumont Hospital Ethics Committee, Dublin, Ireland (REC 21/33) (see **Appendix 1**). Written, informed consent was obtained from all adult participants with decision-making capacity (see **Appendix 2**). A consent declaration was obtained from the Irish HRCDC for adults lacking decision-making capacity (see **Appendix 3**). Parents provided written informed consent for participants younger than 18-years-old. Participants and proxies were consented for off-label use of everolimus.

7.3 Results

Five patients with GATOR1-related epilepsies were treated with everolimus. Baseline clinical and genetic characteristics are presented in **Table 7.1**. The cohort had a mean age of 35.6 years (range 15-49 years). The median number of ASMs taken at the time of everolimus initiation was three. Patients had previously failed 5-16 ASMs. Two patients had failed VNS. The median baseline monthly seizure frequency was 18 (interquartile range [IQR] 58 seizures per month, range 8 to 86 seizures per month).

Four patients had *DEPDC5* variants, and one patient had a *NPRL3* variant. Patients 1, 2, 3 and 7 had pathogenic or likely pathogenic variants in GATOR1 genes. Patient 4 had a *DEPDC5* missense variant, classified as a VUS using ACMG criteria. Following discussion at our epilepsy genetics MDT, we elected to treat Patient 4, given the compatibility of his epilepsy syndrome with the *DEPDC5* phenotype, and the severity of his seizures and peri-ictal psychiatric symptoms.

The median duration of treatment was 12 months (IQR 19.5 months). The median everolimus dose at last review was 12.5mg/day (IQR 5mg/day). Patients 2, 4 and 7 had everolimus serum concentrations greater than 5ng/mL (range 5.1-8.9ng/mL) at the last assessment. Patients 1 and 3 had everolimus serum concentrations less than 5ng/mL but did not tolerate higher doses of everolimus. Patients 1, 2 and 7 were treatment responders: all experienced a greater than 70% MMSF reduction from baseline (range 74.3-86.1%) (**Table 7.2**). All three responders had LoF *DEPDC5* variants. Patient 4 experienced a 43.9% MMSF reduction from baseline after 27 months of treatment. Patient 3 showed no improvement and stopped everolimus after seven months.

Using the CGI-I rating scale, Patient 7 reported that her seizures and quality of life had ‘very much improved.’ Patient 1 rated his overall clinical condition as ‘much improved.’ Patient 4 rated his overall clinical condition as ‘minimally improved.’ Patient 2 reported ‘no change’ in his overall condition, even though he experienced a significant reduction in seizures. He developed psychiatric symptoms after an extended period of seizure freedom and elected to discontinue everolimus. Patient 3 reported that her clinical

condition was ‘minimally worse’ (**Table 7.2**). A summary of each patient’s treatment course is outlined below. Everolimus treatment is ongoing in three of five patients.

Table 7.1 Baseline characteristics of patients with GATOR1-related epilepsies treated with everolimus

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 7
Age (yrs)	49	46	33	35	15
Sex	Male	Male	Female	Male	Female
Age of onset (yrs)	4	8	0.1	3	2
Epilepsy type	Sleep-related hypermotor epilepsy	Sleep-related hypermotor epilepsy	Sleep-related hypermotor epilepsy	Temporal lobe epilepsy	Frontal lobe epilepsy
Active seizure types	Focal unaware (hyperkinetic)	Focal unaware (hyperkinetic)	Focal unaware (hyperkinetic)	Focal unaware (emotional) and focal to bilateral tonic-clonic	Focal unaware (automatisms) and focal to bilateral tonic-clonic
Baseline seizure frequency[a]	86 per month	11 per month	49 per month	18 per month	8 per month
Baseline ASMs	ESL, LTG, PHT, VLP	BRV, PER, CBZ	CBZ, PER, LCM	CBZ, LCM	LCM, ESL, PER
Prior ASMs	CLB, PER, LEV, PGB, ZNS	TPM, LTG, VLP, TGB, LCM, CLB, GBP, LEV, PB, PGB, VIG, ZNS, ESL	FBM, CLB, VIG, PGB, PB, RUF, RTG, ESL, PHT, ZNS, TPM, LEV, LTG, VLP	VLP, CLB, TGB, ESL, PER, TPM, ZNS, GBP, VIG, LTG, LEV, RTG, OXC, PHT, PGB, RUF	CBZ, LEV, VLP, CLB, OXC
Surgery or VNS	No	No	VNS	VNS	No
Gene	*DEPDC5*	*DEPDC5*	*NPRL3*	*DEPDC5*	*DEPDC5*
Variant type	Stop-gain	Frameshift microdeletion	Splicing	Missense	Deletion
ACMG criteria	Pathogenic	Likely pathogenic	Pathogenic	Pathogenic	VUS

Footnotes
[a] Baseline seizure frequency was calculated as the mean number of seizures per month over the previous 3 months before starting everolimus.

Abbreviations:
ACMG= American College of Medical Genetics and Genomics; ASM= anti-seizure medication; BRV= brivaracetam; CBZ= carbamazepine; CLB= clobazam; ESL= eslicarbazepine; FBM= felbamate; GBP= gabapentin; LCM= lacosamide; LEV= levetiracetam; LTG= lamotrigine; OXC= oxcarbazepine; PB= phenobarbital; PER= perampanel; PGB= pregabalin; PHT= phenytoin; RTG= retigabine; RUF= rufinamide; TGB= tiagabine; TPM= topiramate; VIG= vigabatrin; VLP= valproate; VNS= vagus nerve stimulation; VUS= variant of uncertain significance; ZNS= zonisamide

Table 7.2 Everolimus treatment outcomes in GATOR1-related epilepsies

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 7
Duration of treatment	31 months	12 months	7 months	27 months	12 months
Everolimus dose at last review	15mg	10mg	12.5mg	15mg	10mg
Everolimus level at last review	3.2ng/mL	5.1ng/mL	4.2ng/mL	6.2ng/mL	8.9ng/mL
Baseline MMSF	86	11	49.33	18.33	7.66
MMSF at 3 months on treatment	36	4.33	51.33	16.66	2.66
MMSF at 6 months on treatment	21.66	3.66	61.5	11.83	1.5
MMSF at 12 months on treatment	14.58	2.83	-	11.91	1.08
MMSF at 18 months on treatment	11.94	-	-	9.94	-
MMSF at last review on treatment	12.03	2.83	62.57	10.29	1.08
Monthly seizure burden reduction	86.1%	74.3%	No reduction *(26% increase in seizure burden)*	43.9%	85.9%
CGI-I post everolimus	Much improved	No change	Minimally worse	Minimally improved	Very much improved
Treatment-emergent adverse events	Stomatitis (mild)	Stomatitis (moderate), low mood and insomnia (moderate)	Stomatitis (severe)	High serum cholesterol and triglycerides (severe)	Stomatitis (mild), acneiform rash (mild)
Everolimus retention	Yes	No (*stopped after 12 months due to adverse events)*	No *(stopped after 7 months due to lack of efficacy)*	Yes	Yes

Abbreviations:
CGI-I= Clinical Global Impression of Improvement; MMSF= mean monthly seizure frequency

7.3.1 Patient 1: NM_001242896.3(DEPDC5): c.3436C>T (p.Gln1146Ter)

Patient 1 commenced everolimus aged 49 years. Prior to everolimus, he had at least two hyperkinetic seizures with impaired awareness every night. He was treated with phenytoin 225mg/day, eslicarbazepine 800mg/day, lamotrigine 250mg/day and sodium valproate 1200mg/day. After three months, MMSF had reduced from baseline by more than 50%. After 18 months of treatment, the MMSF had reduced by almost 90% and has remained at this frequency over the subsequent 13 months. No other ASMs were added during this period. At last review, he was taking everolimus 15mg/day, with a serum level of 3.2ng/mL. He developed severe stomatitis on everolimus 17.5mg/day, which resolved with dose reduction to 15mg/day. An attempt to withdraw phenytoin led to increased seizures and was aborted.

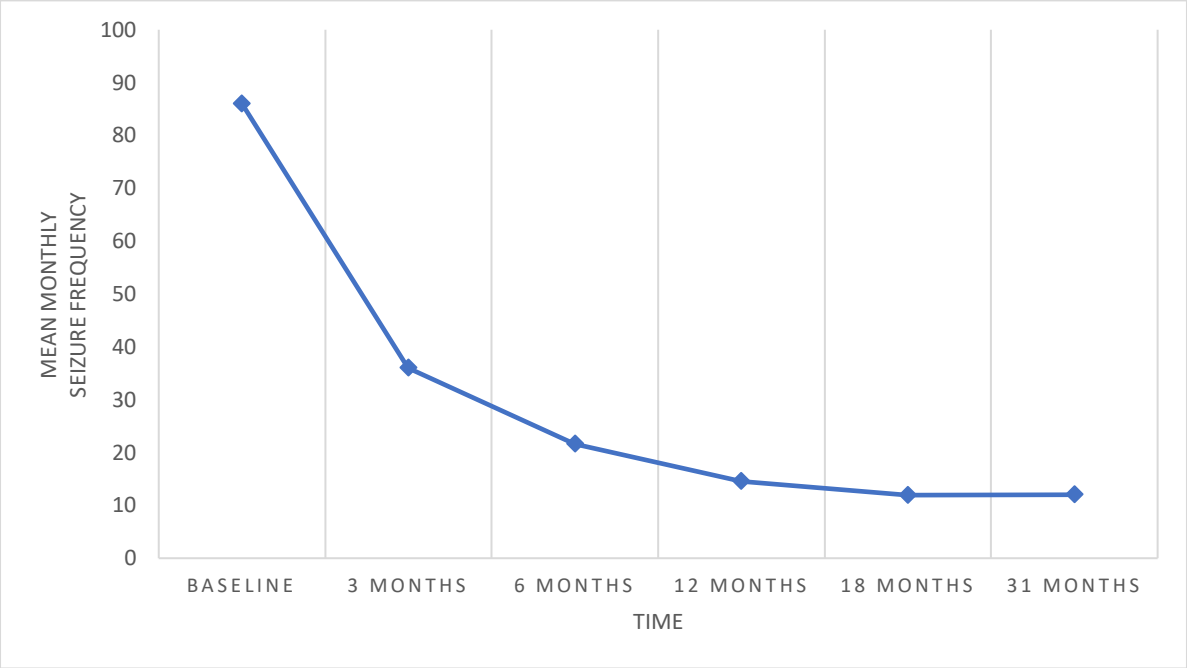

Figure 7.1 Patient 1 seizure frequency trend on everolimus

7.3.2 Patient 2: NM_001242896.3(DEPDC5):c.675del (p.Tyr226fs)

Patient 2 started everolimus aged 46 years. He had sleep-related hypermotor epilepsy since aged eight years. At baseline, he had two to three nocturnal hyperkinetic seizures with impaired awareness per week. He was treated with brivaracetam 275mg/day, carbamazepine prolonged release 1000mg/day and perampanel 6mg/day. After six months on everolimus 10mg/day, the MMSF had reduced by over 70%. The everolimus dose was increased to 12.5mg/day and six weeks of seizure freedom followed. However, he developed low mood and insomnia. These symptoms improved after reducing everolimus to 10mg/day. After 12 months of treatment, the MMSF had reduced by almost 75% but he elected to stop everolimus because of residual psychiatric symptoms and intermittent stomatitis. No other ASMs were added during this period.

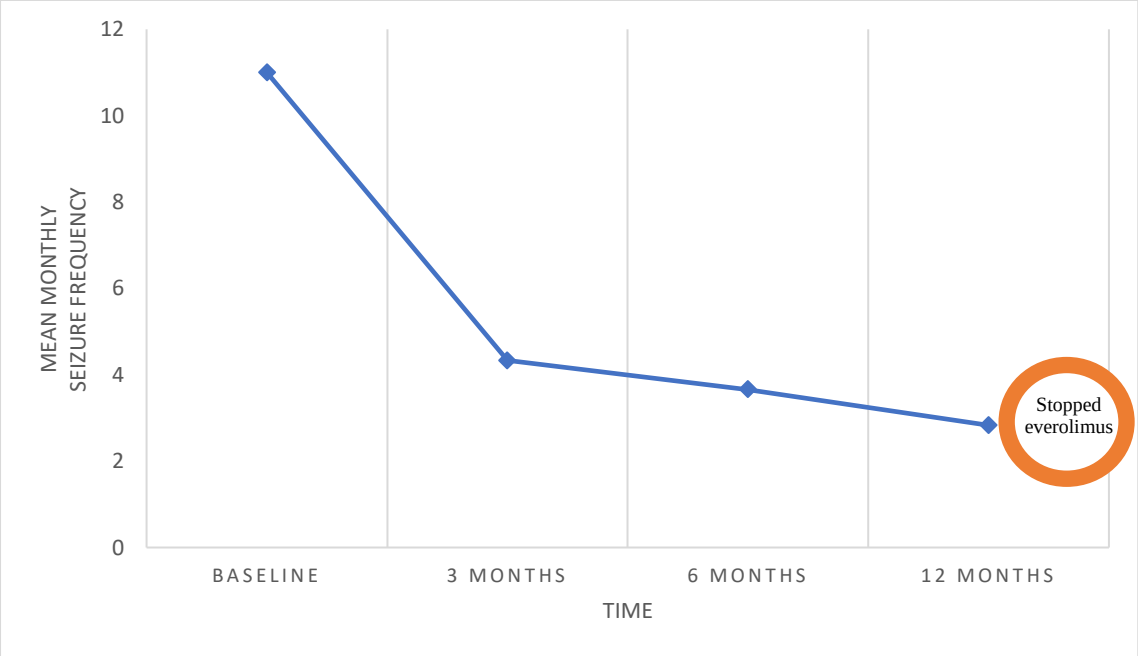

Figure 7.2 Patient 2 seizure frequency trend on everolimus

7.3.3 Patient 3: NM_001077350.3(NPRL3):c.189-1G>A

Patient 3 commenced everolimus aged 33 years. At baseline, she had at least one hyperkinetic seizure with impaired awareness every night. She had a remote history of focal to bilateral tonic-clonic seizures. She was taking carbamazepine prolonged release 1000mg/day, lacosamide 100mg/day and perampanel 4mg/day. She started everolimus 7.5mg/day, with up-titration to 12.5mg/day over six months (serum level of 4.2ng/mL). Stomatitis developed on everolimus 12.5mg/day and was managed by interrupting treatment for three days. After seven months on everolimus, the MMSF had increased from baseline by 26%. She elected to stop everolimus due to lack of efficacy.

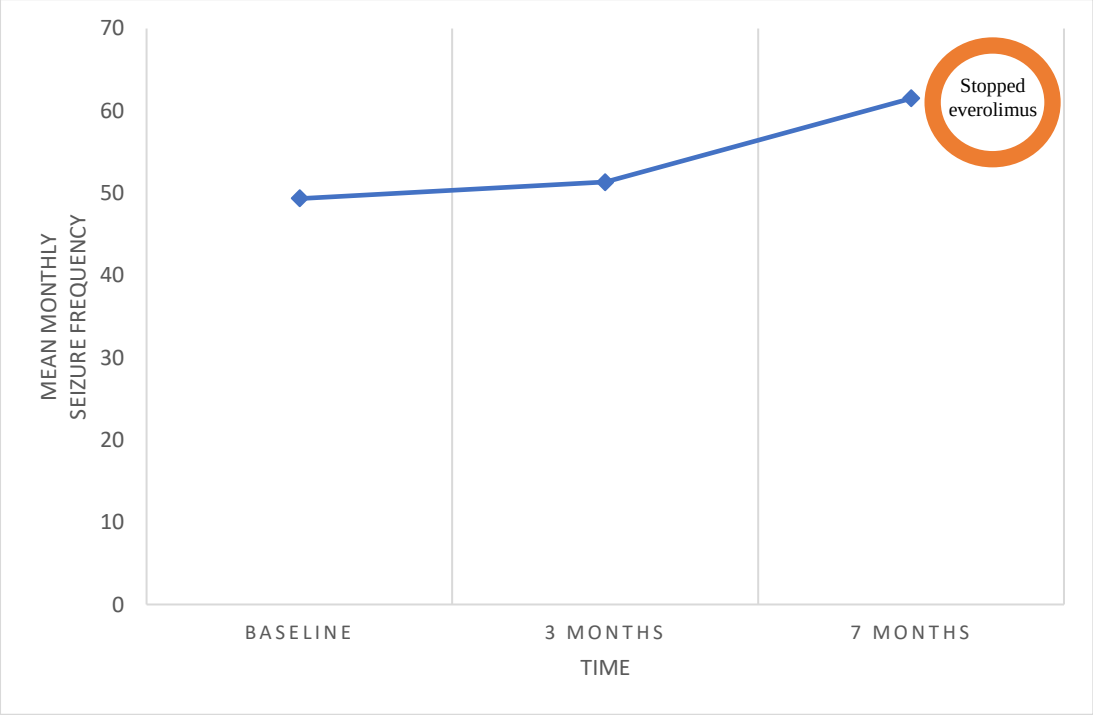

Figure 7.3 Patient 3 seizure frequency trend on everolimus

7.3.4 Patient 4: NM_001242896.3(DEPDC5):c.4283G>A (p.Ser1428Asn)

Patient 4 started everolimus aged 35 years. He had mild ID, verbal dysfluency and severe psychiatric symptoms. Prior to everolimus, he had two to three focal impaired awareness seizures with emotional and cognitive features per week. In addition, he had at least three focal to bilateral tonic-clonic seizures per month. He was treated with lacosamide 400mg/day and carbamazepine prolonged release 1200mg/day. He started everolimus 10mg/day, with up-titration to 15mg/day over six months (serum level of 6.2ng/mL). His MMSF reduced by over 40% after 27 months on everolimus, without changing his concomitant ASMs. The patient and his family also reported improved verbal fluency and reduced psychiatric symptoms since the introduction of everolimus. However, seizures were potentially underreported as he lived alone, and he continued to have at least one focal to bilateral tonic-clonic seizure per month. Fasting serum cholesterol was noted to be significantly raised at 9.8 mmol/L (normal range 0-5 mmol/L) on surveillance blood monitoring. Low-density lipoprotein cholesterol ('bad cholesterol') and fasting triglycerides were also above target range. The patient wanted to try dietary adjustments before trialling a statin.

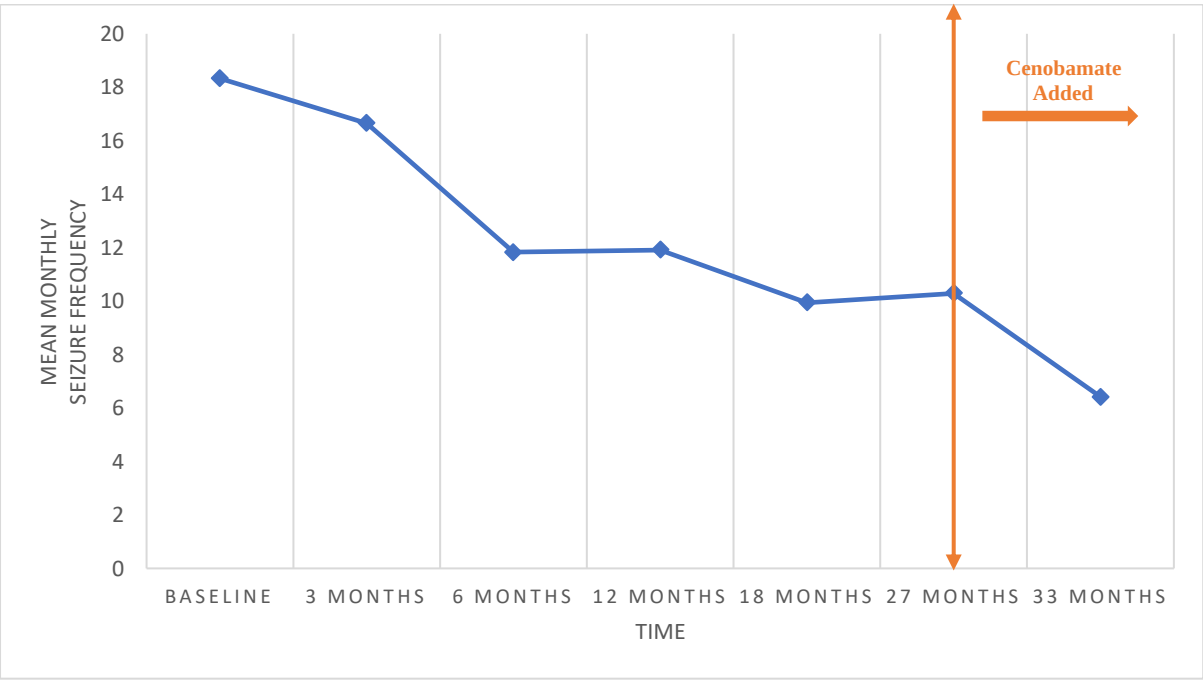

Figure 7.4 Patient 4 seizure frequency trend on everolimus

Following the study period, cenobamate was initiated given his suboptimal seizure control. After six months on cenobamate, his seizure frequency reduced further (greater than 50% reduction) and lacosamide was withdrawn. At recent review, he was taking cenobamate 300mg/day daily and everolimus 15mg/day, with a plan to gradually withdraw carbamazepine.

7.3.5 Patient 7: *DEPDC5* Chr22: g.31796907_31805710del

Patient 7 started everolimus aged 15 years. Before everolimus, she had eight focal-onset seizures with automatisms and impaired awareness per month. These occasionally progressed to bilateral tonic-clonic seizures. She was treated with lacosamide 600mg/day, eslicarbazepine 1200mg/day and perampanel 5mg/day. She started everolimus 7.5mg/day and soon after developed stomatitis and acne. These resolved without adjusting the everolimus dose. After 12 months of treatment, there was an 85% monthly seizure burden reduction, with no focal to bilateral tonic-clonic seizures. No other ASMs were added during this period. At recent review, she was taking everolimus 10mg/day (serum level of 8.9ng/mL), and eslicarbazepine was being gradually withdrawn to reduce her medication burden because of the excellent response to everolimus.

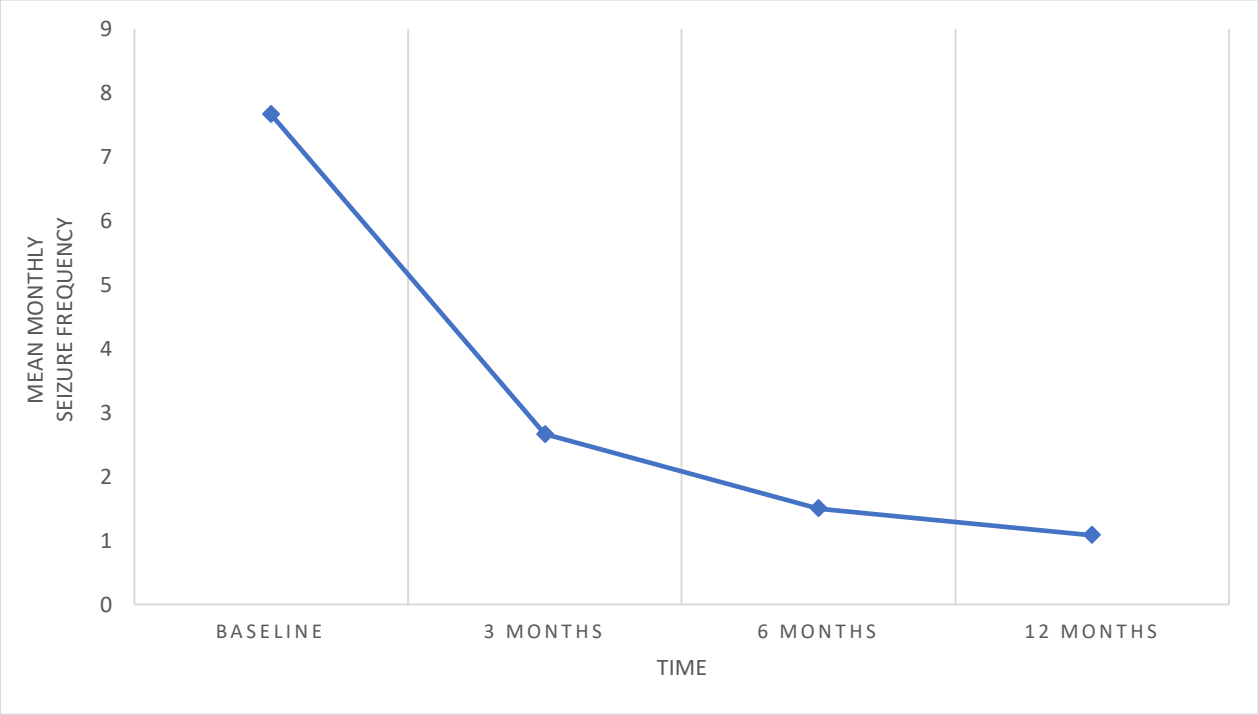

Figure 7.5 Patient 7 seizure frequency trend on everolimus

7.4 Discussion

This pilot observational study presents the first human clinical data on the potential benefit of adjunctive everolimus in GATOR1-related epilepsies. Everolimus treatment led to significant seizure frequency reductions in three patients with LoF *DEPDC5* variants. Seizure improvements increased over time, supporting the hypothesis that mTOR inhibitors address the underlying pathophysiology of epileptogenicity in *DEPDC5*-related epilepsies. The patient with a *DEPDC5* missense variant had reduced seizures on everolimus, although the magnitude of improvement was less compared to patients with LoF *DEPDC5* variants. Everolimus was ineffective at reducing seizures in the patient with a *NPRL3* splice-site variant.

We postulated that the anti-seizure properties of mTOR inhibitors observed in TSC may also be applicable to the GATOR1-related epilepsies and possibly other mTORopathies[136]. This theory is supported by studies demonstrating rapamycin-responsive seizures in mouse models of *Depdc5, Nprl2* and *Nprl3* knockout[262, 263], and molecular evidence of mTORC1 hyperactivation in brain resections from patients with *DEPDC5* and *NPRL3* pathogenic variants[131, 134, 278]. All patients treated with everolimus had highly active (median baseline seizure frequency of 18 per month) and refractory focal epilepsy (median of 13 failed ASM trials). All five patients had been considered for epilepsy surgery but either declined intracranial EEG monitoring or were deemed unsuitable for surgery due to multifocal epilepsy. Given the severity of the patients' epilepsy and the mechanistic rationale for mTOR inhibitor targeted treatment, we undertook this off-label everolimus study following ethics committee approval.

The most substantial seizure frequency reductions were observed in three patients with LoF *DEPDC5* variants predicted to cause NMD, haploinsufficiency and loss of mTORC1 inhibition. A number of studies have provided proof for a LoF mechanism with truncating *DEPDC5* and *NPRL3* variants, with mTOR hyperactivation the functional consequence, and likely cause of seizures[131-134]. In the largest GATOR1-related epilepsy series, over two-thirds of patients had LoF variants[127]. Patients with LoF GATOR1 variants had higher rates of FCD, epileptic spasms and SUDEP

compared to those with missense variants, suggesting a more severe phenotype with LoF variants[127]. For the reasons outlined above, patients with LoF GATOR1 variants should be targeted for future mTOR inhibitor trials.

Patient 3 derived no clinical benefit from everolimus. Potential reasons for treatment failure included the below target serum everolimus level and the long history (early onset infantile spasms) of highly active epilepsy. Genetic factors may have also contributed, including the causal gene (*NPRL3*) and variant type (splice-site). The anti-seizure benefit of rapamycin was significantly less durable after treatment withdrawal in *Nprl3* knockout mice compared with *Depdc5* knockout mice, suggesting that *NPRL3*-related epilepsy may be less responsive to mTOR inhibitor treatment compared to *DEPDC5*-related epilepsy[263]. Two case reports of sirolimus for DRE in the setting of *NPRL3*-related HME provided mixed findings. One infant had significantly reduced seizures but discontinued treatment after 3.5 months due to recurrent infections[337]. The other infant stopped sirolimus after 17 days due to lack of efficacy[338].

We elected to treat Patient 4 following MDT discussion. Despite some improvement in seizure control, he continued to have frequent focal to bilateral tonic-clonic seizures. Notwithstanding our rationale for treating this patient, there are limits to the conclusions that can be drawn given the variant's classification as a VUS. Functional assessments of many *DEPDC5* missense variants have failed to provide evidence of mTORC1 hyperactivation[271]. *DEPDC5* missense variants likely have less impact on mTORC1 activation compared with LoF variants, and may result in seizures through mTORC1-independent mechanisms[136]. Potential anti-seizure and anti-epileptogenic effects of mTOR inhibitors were observed in rodent models of non-TSC-related epilepsies, and these may explain the partial treatment response in this patient[418]. It is also possible that the seizure frequency reduction occurred spontaneously and was unrelated to everolimus treatment.

Four of five patients developed stomatitis. Stomatitis resolved spontaneously in Patient 7, following dose reduction in Patient 1, and following interruption of treatment in Patient 3. Poor tolerability at higher everolimus doses meant two patients (Patient 1 and 3) did not attain the target everolimus serum level, although Patient 1 still had

significantly reduced seizures at a serum level of 3.2ng/mL. Similarly, many participants in the EXIST-3 trial failed to achieve the target serum everolimus level[135]. No patients reported increased infection rates after starting everolimus. One patient developed significant hypercholesterolemia and hypertriglyceridemia. No cytopenias were detected on surveillance blood testing.

Recurrent stomatitis and psychiatric symptoms contributed to treatment discontinuation in Patient 2. He developed low mood and insomnia after a period of six weeks without seizures. As psychiatric symptoms are a very uncommon complication of everolimus treatment, we felt their emergence in this case represented "forced normalisation" in the context of significantly improved seizure control[419]. Forced normalisation is a well described phenomenon characterised by the emergence of psychiatric symptoms (psychosis, depression, mania, anxiety or dissociation) following the establishment of seizure freedom in patients with previously uncontrolled epilepsy. Forced normalisation can be induced by ASMs, epilepsy surgery or VNS[419].

A number of study limitations warrant mentioning. First, the small sample size and open-label observational study design made meaningful quantitative analysis difficult, and conclusions tentative and preliminary. Second, the use of self-report seizure diaries may have led to seizure underreporting or reporting of non-seizure events. Third, the functional effects of GATOR1 genetic variants were not tested. The issues related to including a patient with a VUS were discussed earlier. Fourth, two participants did not reach the target serum everolimus concentration due to dose-dependent complications.

Notwithstanding these limitations these findings suggest that everolimus is a promising targeted treatment for *DEPDC5*-related DRE. Epilepsy patients with putative, or preferably functionally characterised LoF variants in GATOR1 genes should be targeted for future mTOR inhibitor clinical trials. Large, randomised placebo-controlled studies may not be feasible due to the rarity of the GATOR1-related epilepsies, although more routine genetic testing in patients with refractory focal epilepsy may increase numbers for study. '*N-of-some*' trials involving participants recruited from well organised clinical networks of patients with pathogenic variants in

the same gene may facilitate larger precision therapy trials. Stomatitis was a dose-limiting complication and contributed to treatment discontinuation in one patient. Brain-selective ATP-competitive mTOR kinase inhibitors are under development and could represent a less systemically toxic therapeutic option[365].

In conclusion, this observational study suggests that everolimus can reduce seizures in DRE caused by LoF variants in *DEPDC5.* Larger studies are needed to confirm this finding. It remains to be determined if mTOR inhibitors can effectively treat seizures in epilepsies caused by variants in *NPRL2* and *NPRL3,* or *DEPDC5* missense variants. Indeed, further work is required to understand the pathogenicity of GATOR1 missense variation. In the next chapter I review the evidence supporting the efficacy of epilepsy surgery for GATOR1-related epilepsies and other monogenic epilepsies.

8. Epilepsy surgery for refractory mTOR-related epilepsies and other monogenic epilepsies: a scoping review

8.1 Introduction

In focal DRE, epilepsy surgery offers a greater chance of achieving seizure freedom, compared with continued ASM trials[68]. The best candidates for epilepsy surgery have visible lesions on MRI ('MRI positive') and concordant ictal vEEG data. For example, surgery for temporal lobe epilepsy caused by mesial temporal sclerosis (MTS) results in seizure freedom in approximately 70% of cases[69]. MTS (also known as hippocampal sclerosis) is characterised radiologically by hippocampal atrophy and signal hyperintensity on T2-weighted MRI, and hippocampal neuronal cell loss and gliosis on neuropathological examination. Prolonged early life febrile convulsions are an important risk factor for later MTS. The FEBSTAT study demonstrated that many children with prolonged febrile seizures developed MTS on follow-up MRI[420].

Epilepsy surgery outcomes are less impressive in extra-temporal epilepsies, particularly if high-resolution MRI is normal ('MRI negative'). The application of intracranial EEG improves outcomes in extra-temporal epilepsies, with seizure freedom rates approaching 60% for frontal lobe resections following presurgical stereoelectroencephalography (SEEG)[70]. SEEG involves intracranial surgical implantation of depth electrodes to better localise seizure foci. Other non-invasive modalities such as FDG-PET brain, ictal SPECT and high-density EEG can help localise seizure-onset zones.

The epilepsy surgery review meeting (ESRM) facilitates systematic multidisciplinary review of presurgical data. Epileptologists, neurosurgeons, neurophysiologists, neuropsychologists, neuropsychiatrists and nurse specialists systematically review epilepsy surgery candidates' seizure history and semiology, vEEG data, structural and metabolic imaging, and neuropsychological and psychosocial profile. Surgical candidacy is determined by consensus based on concordant findings and a surgical

strategy is formulated, including the possible requirement for intracranial EEG (see **Figure 8.1**).

Exome sequencing is increasingly performed as part of the presurgical evaluation at Beaumont Hospital. This aligns with the practice of other advanced epilepsy centres[421, 422]. Patients with focal non-lesional epilepsy and epilepsy with co-morbid ID are prioritised for genetic testing during the presurgical evaluation. Genetic testing may also be indicated for patients with focal epilepsy due to MCD or other genetically mediated brain lesions. We evaluate the clinical significance of candidate genetic variants at the epilepsy genetics MDT meeting. Relevant genetic data is then presented at the ESRM to assist decision-making about surgical candidacy and strategy (see **Figure 8.1**).

This literature review aims to identify and summarise genetic data that can inform predictions on epilepsy surgery candidacy and outcomes. This study expands on Stevelink *et al*'s work on epilepsy surgery outcomes in patients with monogenic epilepsies[423]. Findings from their systematic review suggest that focal epilepsies caused by mutations in mTOR genes could be improved or cured with epilepsy surgery, whereas *SCN1A*-related epilepsies generally have less favourable surgical outcomes[423]. I also review the emerging contribution of somatic mosaicism in focal epilepsies and consider implications for surgical management. The findings from this literature review are reported in Moloney *et al*[117].

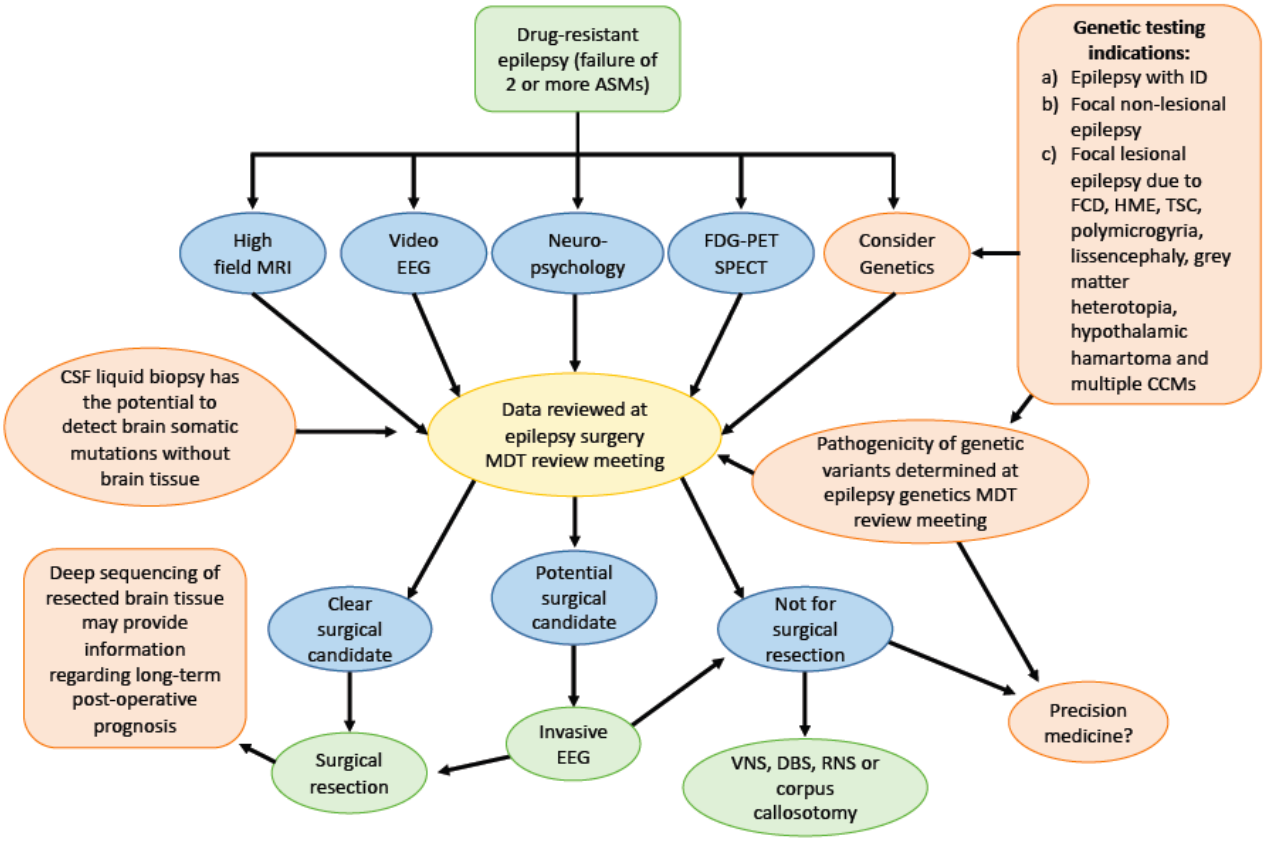

Figure 8.1 A conceptual framework embedding genomic data in the epilepsy surgery evaluation. The established model involves the acquisition of vEEG, high-resolution MRI, metabolic imaging and neuropsychological data. Data is presented systematically at a multidisciplinary meeting, where surgical candidacy is determined by consensus, including the requirement for invasive EEG monitoring. The inclusion of appropriate genetic testing in the presurgical evaluation could improve predictions on surgical candidacy, identify patients with 'MRI invisible' dysplasia who might benefit from invasive EEG monitoring and promotes early adoption of precision therapies when available. The clinical significance of candidate genetic variants should be evaluated at a multidisciplinary meeting attended by clinical geneticists and bioinformaticians. Postoperative analysis of resected brain tissue may provide useful prognostic information.

Abbreviations: CCM= cerebral cavernous malformation; CSF= cerebrospinal fluid; DBS= deep brain stimulation; FCD= focal cortical dysplasia; FDG-PET= *fluorodeoxyglucose*- positron emission tomography; HME= hemimegalencephaly; RNS= responsive neurostimulation; SPECT= single photon-emission computed tomography; VNS= vagus nerve stimulation.

8.2 Methods

Peer-reviewed articles reporting epilepsy surgery outcomes in patients with germline monogenic epilepsies were identified by three methods:

e) Search of PubMed [1993-2022] using the search terms ([epilepsy] OR [seizure]) AND ([genetic] OR [mutation] OR [monogenic]) AND ([epilepsy surgery]). *Search date November 14th, 2022; 176 results*.
f) Previous literature searches on GATOR1-related epilepsies and other mTORopathies.
g) Review of references in articles identified from the PubMed search.

Search and study selection strategies are summarised in **Figure 8.2**. Patients with epilepsy caused by TSC, familial cerebral cavernous malformations (CCM) and other diffuse bihemispheric brain lesions (for example, lissencephaly and polymicrogyria) were excluded from the systematic review. First, I reviewed the titles and abstracts of articles potentially eligible for inclusion. If titles or abstracts were deemed to be potentially relevant, the full texts were then reviewed. I manually searched the references of selected articles to identify additional pertinent manuscripts.

Data extracted from eligible articles included: the causal gene; number of epilepsy surgery patients; histology of resected tissue; MRI findings; surgery type; and surgical outcome. Engel Class I (free of disabling seizures) and Engel Class II (rare disabling seizures) were considered favourable surgical outcomes.

Lastly, I conducted a narrative review on epilepsy surgery for epilepsies caused by monogenic diffuse MCD and familial CCM, as well as surgery for focal epilepsies caused by somatic mutations.

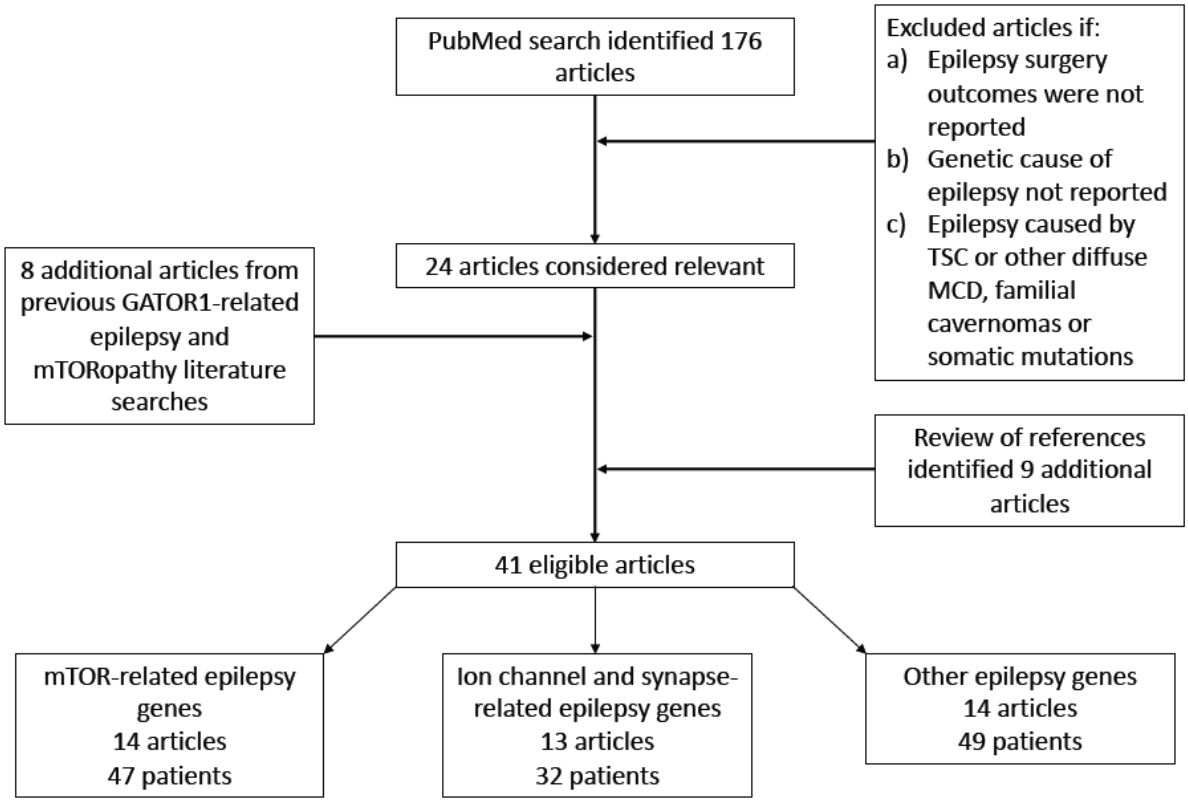

Figure 8.2 Flow chart of search strategy and study selection

8.3 Results

8.3.1 Surgical outcomes in epilepsies caused by germline mTOR gene mutations

Stevelink *et al*'s systematic review identified 12 patients with germline mTOR gene mutations who underwent epilepsy surgery[131, 154, 235, 278, 407, 423]. An additional 35 epilepsy patients with mTOR gene mutations who underwent epilepsy surgery were identified from four research studies[90, 127, 158, 421], two case series[422, 424], and three case reports[337, 338, 425] published since 2018. Surgical outcomes for the 47 epilepsy patients with mTOR pathway mutations are presented in **Table 8.1**. Overall, 63.8% (30/47) of patients had Engel Class I or Class II surgical outcomes. Thirty patients (63.8%) had pathogenic variants in *DEPDC5,* 12 (25.5%) had *NPRL3* pathogenic variants, three (6.4%) had *NPRL2* pathogenic variants, one patient (2.1%) had a pathogenic variant in *PTEN,* and one patient (2.1%) with FCD type II had a germline variant in *TSC2*. Patients with FCD or HME ('MRI positive') had similar surgical outcomes (64.1% Engel I or II) to 'MRI negative' patients (62.5% Engel I or II), although only eight patients with normal MRI were identified and included in the analysis. Four out of eight (50%) patients with normal MRI had histological evidence of FCD. Poor surgical outcomes were observed in a series of four children with familial GATOR1-associated FCD. All had a more severe phenotype than their parents, suggestive of a possible phenotypic gradient from affected parents to offspring[424].

Table 8.1 Epilepsy surgery outcomes in patients with germline mutations in mTOR pathway genes

	Study description	MRI-positive	MRI-negative	Total group	Reference
1	Systematic review of studies reporting epilepsy surgery outcomes in paediatric and adult patients with monogenic epilepsies	7/8 (87.5%) patients had favourable surgical outcomes (*DEPDC5* n=6; *NPRL3* n=1; *PTEN* n=1)	2/4 (50%) patients had favourable surgical outcomes (*DEPDC5* n=3; *NPRL2* n=1)	9/12 patients had favourable surgical outcomes (75%)	423
2	Phenotyping study of paediatric and adult patients with GATOR1 mutations	6/8 (75%) patients had favourable surgical outcomes (*DEPDC5* n=6; *NPRL2* n=1; *NPRL3* n=1)	2/2 (100%) patients had favourable surgical outcomes (*DEPDC5* n=2)	8/10 patients had favourable surgical outcomes (80%)	127
3	Clinical and genetic analysis of a paediatric epilepsy surgery cohort with FCD or HME	4/6 (66.66%) patients had favourable surgical outcomes (*DEPDC5 n*=5; *TSC2* n=1)	-	4/6 patients had favourable surgical outcomes (66.66%)	90
4	Clinical and genetic analysis of a paediatric epilepsy surgery cohort with bottom-of-sulcus dysplasia	1/2 (50%) patients had favourable surgical outcomes (*DEPDC5* n=1; *NPRL3* n=1)	-	1/2 patients had favourable surgical outcomes (50%)	158
5	A retrospective single-centre study on the clinical utility of genetic testing in children and adults who underwent epilepsy surgery	1/1 (100%) patients had favourable surgical outcomes (*NPRL3* n=1)	1/2 (50%) patients had favourable surgical outcomes (*DEPDC5* n=2)	2/3 patients had favourable surgical outcomes (66.66%)	421
6	A case series of children who underwent epilepsy surgery for FCD-related epilepsy caused by GATOR1 mutations	0/4 patients had favourable surgical outcomes (*DEPDC5* n=2; *NPRL2* n=1; *NPRL3* n=1)	-	0/4 patients had favourable surgical outcomes	424
7	A case series of children and adults with monogenic epilepsies who underwent epilepsy surgery	3/7 (42.9%) patients had favourable surgical outcomes (*DEPDC5* n=3; *NPRL3* n=4)	-	3/7 patients had favourable surgical outcomes (42.9%)	422

8	Case report of infant with *NPRL3*-related FCD who underwent temporal lobectomy following stereo-EEG investigation	1/1 (100%) achieved favourable surgical outcome	-	1/1 (100%) achieved favourable surgical outcome	425
9	Case report of infant with *NPRL3*-related HME who underwent functional hemispherotomy	1/1 (100%) achieved favourable surgical outcome	-	1/1 (100%) achieved favourable surgical outcome	337
10	Case report of infant with *NPRL3*-related HME who underwent functional hemispherotomy	1/1 (100%) achieved favourable surgical outcome	-	1/1 (100%) achieved favourable surgical outcome	338
Total	**-**	**25/39 (64.1%)**	**5/8 (62.5%)**	**30/47 (63.8%)**	**-**

Footnotes:
Surgical success defined as Engel Class I or II.

Abbreviations:
FCD= focal cortical dysplasia; HME, hemimegalencephaly.

8.3.2 Surgical outcomes in channelopathies and synaptopathies

The epilepsy surgery outcomes of 18 patients with *SCN1A* mutations were reported in four case series[147, 422, 426, 427]. Favourable seizure outcomes were observed in 27.7% of cases overall (see **Table 8.2**). All patients (n=12) with electroclinical features consistent with Dravet syndrome had poor surgical outcomes, including cases with MTS and FCD. Neuropathological findings in patients with *SCN1A*-related MCD comprised mild MCD, FCD type I and FCD type II[147, 426, 427].

Five patients with *SCN1A*-related unifocal epilepsy lacking the Dravet phenotype benefited from resective surgery[426]. Four patients had mesial temporal ictal EEG onsets and concordant MTS on MRI, and one patient had seizures arising from the occipital lobe and normal MRI. All had febrile seizures as infants. Thus, epilepsy surgery may benefit a subset of *SCN1A*-related epilepsies with unilateral focal seizures and concordant MRI data, while patients with the multifocal and generalised EEG onsets seen in Dravet syndrome rarely benefit from surgery (see **Table 8.2**). Palliative procedures like corpus callosotomy, VNS and DBS provided modest seizure reduction in some Dravet syndrome patients[428-430].

Three patients with 'MRI negative' *KCNT1*-related focal epilepsies had persistent seizures after resective epilepsy surgery[148]. Neuropathological analysis in all cases revealed FCD type I, which may reflect a more diffuse cortical malformation in association with *KCNT1* mutations. A patient with *SCN8A*-related epilepsy and normal MRI underwent right anterior temporal lobectomy, after SEEG localised seizures to the right hippocampus. Epilepsy surgery resulted in a greater than 50% reduction in seizures[431]. Pathogenic *SCN1B* variants are associated with GEFS+ and temporal lobe epilepsy. Surgical outcomes were favourable in *SCN1B*-related epilepsy compared with other channelopathies. Two patients with unilateral temporal lobe epilepsy and concordant EEG data underwent temporal lobectomy. One patient had MTS and the other had normal brain imaging. Both were seizure-free after two years follow-up[432].

Palliative procedures like VNS and corpus callosotomy may be beneficial in DEE caused by mutations in ion channel genes. For example, VNS led to modest seizure reductions in patients with pathogenic variants in *SCN8A*[433, 434], while corpus

callosotomy improved seizure and developmental outcomes in a child with a *KCNQ2* mutation[435].

Pathogenic variants in genes involved in regulation of synaptic transmission (for example, *STXBP1, CNTNAP2* and *STX1B)* produce severe epilepsies, often as DEE. Epilepsy surgery reduced seizures in three *STXBP1* DEE patients[151, 436, 437]. One of these cases had normal brain imaging and right occipitotemporal EEG abnormalities; complete occipital disconnection and multiple temporal subpial transections reduced seizures by 95%[436]. The second case had FCD on MRI; lesionectomy achieved complete seizure remission after two years. A second-hit mosaic mutation in *STXBP1* was detected in resected brain tissue[151]. Neuropathological findings were consistent with FCD type I in both cases[151, 436]. The third patient had West syndrome with no epileptogenic lesion detected on MRI; corpus callosotomy led to seizure freedom[437]. Epilepsy surgery for refractory *CNTNAP2*-related DEE (n= 4) and refractory *STX1B*-related temporal lobe epilepsy (n= 1) failed to control seizures, including cases with focal MRI and video EEG findings. Neuropathological findings comprised FCD type I in *CNTNAP2*-related epilepsy and mild MCD in *STX1B*-related epilepsy[150, 421, 438].

Table 8.2 Epilepsy surgery outcomes in patients with *SCN1A* mutations

	Study description	Unifocal epileptiform discharges	Multifocal or generalised epileptiform discharges	Total group	Reference
1	A case series of children and adults with *SCN1A* mutations who underwent epilepsy surgery	-	0/6 patients had favourable surgical outcomes *(Normal MRI and pathology n=4; MTS/ HS n=1; traumatic gliosis n=1)*	0/6 patients had favourable surgical outcomes	427
2	A case series of paediatric patients with *SCN1A* mutations and MCD	-	*0/2* patients had favourable surgical outcomes *(FCD type I n=1; FCD type II n=1)*	0/2 patients had favourable surgical outcomes	147
3	A case series of children and adults with *SCN1A* mutations who underwent epilepsy surgery	5/5 (100%) patients had favourable surgical outcomes *(MTS/ HS n=4; normal MRI and pathology n=1)*	0/3 patients had favourable surgical outcomes *(FCD type I n=1; FCD unspecified n=1; MTS/ HS n=1)*	5/8 (62.5%) patients had favourable surgical outcomes	426
4	A case series of children and adults with monogenic epilepsies who underwent epilepsy surgery	0/1 patients had favourable surgical outcomes *(MTS/ HS n=1)*	0/1 patients had favourable surgical outcomes *(FCD type II n=1)*	0/2 patients had favourable surgical outcomes	422
Total	**-**	**5/6 (83.3%)**	**0/12 (0%)**	**5/18 (27.7%)**	

Footnotes:
Surgical success was categorised as Engel Class I or Class II.

Abbreviations:
FCD= focal cortical dysplasia; HS= hippocampal sclerosis; MCD= malformation of cortical development; MTS= mesial temporal sclerosis.

8.3.3 Epilepsy surgery for other monogenic epilepsies

Pathogenic variants in *PCDH19* produce the distinctive phenotype of female restricted epilepsy with ID. This early infantile epilepsy syndrome with prominent fever-sensitive seizures resembles Dravet syndrome. *PCDH19* encodes a protocadherin involved in cell-cell adhesion[439]. Radiological evidence of FCD was observed in a cohort of female children with *PCDH19* mutations, of whom two underwent epilepsy surgery for DRE. Both experienced significant seizure frequency reductions, but neither achieved seizure freedom. Neuropathological analysis demonstrated FCD type I in both cases[149]. A two-year-old female patient with *PCDH19*-related epilepsy underwent left temporal lobectomy. She had left temporal ictal onsets on vEEG, and MRI brain showed subtle hyperintensity in the left temporal pole. Postoperatively, she experienced a greater than 50% reduction in seizures. Histopathological examination exhibited features of both FCD type I and mild MCD[440].

COL4A1 and *COL4A2* encode for components of type IV collagen, a basement membrane constituent of vascular endothelia and other tissues[441]. *COL4A1/2* mutations are associated with an increased risk of prenatal intracerebral haemorrhage and a broad spectrum of lesions including periventricular leukoencephalopathy, porencephaly, schizencephaly and polymicrogyria[441, 442]. Epilepsy occurs in over 40% of patients with *COL4A1/ 2* mutations[442]. Epilepsy was associated with a structural lesion (for example, porencephalic cyst or FCD) in 46% of cases. All patients with focal cortical MRI abnormalities had additional background leukoencephalopathy[442]. Three children with *COL4A1* mutations underwent epilepsy surgery without haemorrhagic complication. A child with *COL41A*-related FCD became seizure-free following lesionectomy[442]. Functional hemispherectomy significantly reduced seizure frequency in a child with bilateral periventricular leukoencephalopathy and continuous right hemispheric epileptiform discharges[443]. Corpus callosotomy reduced tonic seizures in a child with multifocal, bilateral MRI and EEG abnormalities[444]. *COL4A1/ 2* mutations do not preclude epilepsy surgery in selected cases. Presurgical MRI brain with susceptibility-weighted imaging (SWI) to screen for cerebral microbleeds could assist with quantification of intraoperative haemorrhagic risk. Preoperative screening for multisystem *COL4A1/2* manifestations, including cardiac arrhythmia and renal dysfunction should be performed to reduce perioperative risk[442].

Neurofibromatosis type 1 (NF1) is the most common neurocutaneous syndrome, caused by LoF mutations in *NF1* (neurofibromin). The prevalence of epilepsy in NF1 is ~5.4%. Structural lesions were identified in over half of epilepsy cases, with low-grade glioma, MTS, FCD and dysembryoplastic neuroepithelial tumour (DNET) most frequently encountered[445]. Stevelink *et al's* systematic review found that epilepsy surgery for MTS in NF1 led to seizure freedom in two-thirds of cases[423]. Less favourable epilepsy surgery outcomes were observed in NF1 patients with dual pathology, including cases with MTS and histopathologically proven FCD not detected on presurgical MRI[161, 446]. Similar to mTORopathies, epileptogenic lesions in NF1 can respond to resective surgery.

Recurrent genomic microdeletions (for example, 16p13.11 and 15q11.2) are both potential causes of and risk factors for "common" sporadic epilepsies. In a cohort of patients with microdeletions who underwent epilepsy surgery, all cases with MTS (n=8) achieved seizure freedom[447]. Thus, a genomic microdeletion is not a contraindication to epilepsy surgery, as favourable seizure outcomes can be achieved if MRI and EEG data are concordant. Epilepsy surgery outcomes for monogenic epilepsies, not including those caused by mutations in mTOR pathway genes and *SCN1A*, are outlined in **Table 8.3**.

Table 8.3 Epilepsy surgery outcomes in monogenic epilepsies, excluding mTORopathies and *SCN1A*-related epilepsies

Genetic cause	Mutated gene function	Associated lesion(s)	Number of patients	Engel Class I-II outcome n, (%)	References
Germline *KCNT1* mutation	Ion channel gene	FCD type I	3	0 (0%)	148
Germline *SCN1B* mutation	Ion channel gene	MTS, non-lesional	2	2 (100%)	432
Germline *SCN8A* mutation	Ion channel gene	Non-lesional	1	0 (0%)	431
Germline *STXBP1* mutation	Synaptic transmission gene	FCD type I, non-lesional	3	2 (66.66%)	151, 436, 437
Germline *CNTNAP* mutation (recessive)	Synaptic transmission gene	FCD type I/ mild MCD, MTS	4	0 (0%)	150, 421
Germline *STX1B* mutation	Synaptic transmission gene	Mild MCD	1	0 (0%)	438
Germline *PCDH19* mutation	Cell-cell adhesion gene	FCD type I	3	2 (66.66%)	149, 440
Germline *ARX* mutation (recessive)	Neuronal migration gene	Polymicrogyria	1	0 (0%)	421
Germline *CDKL5* mutation	Neuronal migration gene	Non-lesional	1	0 (0%)	422
Germline *COL41A* mutation	Type IV collagen gene	FCD, leukoencephalopathy	3	1 (33.33%)	442-444
Germline *NF1* mutation	Ras inhibitor gene	FCD, MTS, polymicrogyria, DNET, ganglioglioma, non-lesional	25	13 (52%)	161, 421-423
Germline *ANKRD11* mutation (KBG syndrome)	Chromatin regulator gene	FCD	1	0 (0%)	421
Germline *NSD1* mutation (Sotos overgrowth syndrome)	Transcription regulator gene	Astrocytoma	1	0 (0%)	448

Germline *WDR26* mutation	Not known	Non-lesional	1	0 (0%)	421
Ring chromosome 20 syndrome	Not applicable (chromosomal rearrangement)	Non-lesional	1	0 (0%)	422
Genomic microdeletions (16p13.11 [n=5], 17p12 [n=2], 15q11.2 [n=2], 4q32.3 [n=1], 4q35.2 [n=1], 7q31.32-31-33 [n=1])	Not applicable (genomic microdeletions)	MTS, hamartoma, non-lesional	12	9 (75%)	447, 449

Abbreviations:
DNET= dysembryoplastic neuroepithelial tumour; FCD= focal cortical dysplasia; MCD= malformation of cortical development; MOGHE= Mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy; MTS= mesial temporal sclerosis.

8.3.4 Epilepsy surgery for monogenic bihemispheric malformations of cortical development and familial cerebral cavernous malformations

Monogenic causes of extensive bihemispheric MCD are well described. Grey matter heterotopias are collections of cortical neurons in abnormal locations in the brain, which result from disrupted neuronal migration during embryogenesis. *FLNA* mutations cause bilateral periventricular nodular heterotopia (PVNH) in females, with DRE occurring in 82% of cases[450]. Coexisting MTS is common with PVNH [451]. Temporal lobectomy failed to control seizures in all patients with bilateral PVNH and unilateral temporal epileptiform discharges (n= 7)[452]. Genetic testing was not performed in this study, but some patients likely had genetic MCD as almost half with bilateral PVNH have *FLNA* mutations[453]. Surgery or radiofrequency ablation may be successful in cases with unilateral heterotopia and concordant EEG data, although *FLNA* mutations are almost exclusively associated with bihemispheric abnormalities[454].

Polymicrogyria is a MCD characterised by excessive small folds (gyri) in the brain, leading to abnormally thick cerebral cortex. This highly epileptogenic lesion is associated with early life epilepsy in 80% of cases[455]. Intrauterine cytomegalovirus infection and perinatal ischaemia cause localised polymicrogyria[456]. Germline genetic causes of polymicrogyria produce more extensive bihemispheric abnormalities, with mTORopathies (for example, *PIK3R2* and *PTEN* mutations) and tubulinopathies (for example, *TUBA1A* and *TUBB2B* mutations*)* most commonly implicated[166-168]. Epilepsy surgery can be successful if the epileptogenic zone is carefully delineated by intracranial EEG investigation, particularly in cases with unilateral polymicrogyria[457]. Less favourable surgical outcomes would be expected for genetically mediated polymicrogyria due to more widespread brain involvement. However, genetic analyses were not included in studies on the surgical outcomes of polymicrogyria-related epilepsy[456, 457]. In a series of 26 patients who underwent surgery for polymicrogyria-related epilepsy, seven patients had brain mosaic duplication of the long arm of chromosome 1 (1q) and a specific genomic DNA methylation profile[169]. Patients with brain somatic 1q trisomy had unilateral frontal or hemispheric polymicrogyria. Five out of seven patients achieved postoperative seizure freedom, indicating that this phenotype-genotype correlation may be associated with good surgical outcomes[169].

Lissencephaly comprises a spectrum of MCD including agyria (absence of gyri), pachygyria (broad gyri) and subcortical band heterotopia (also known as double cortex syndrome)[98]. Pathogenic variants in *DCX* are inherited in an X-linked manner and are associated with anterior-predominant lissencephaly. Hemizygous males manifest frontoparietal severe lissencephaly, while heterozygous females display anterior subcortical band heterotopia. *LIS1* mutations are the most common cause of posterior-predominant lissencephaly spectrum disorders[98]. Resective epilepsy surgery was unsuccessful in seven out of eight patients with double cortex syndrome and a localised epileptogenic focus[458]. No patients had genetic testing performed at the time of the study.

Corpus callosotomy was used successfully in a series of ten patients with bihemispheric MCD (for example, lissencephaly and perisylvian polymicrogyria) and represents a palliative surgical option in patients with DRE due to extensive genetically mediated MCD[459]. As an alternative to epilepsy surgery, neuromodulation procedures like VNS and responsive neurostimulation have shown efficacy for DRE due to bihemispheric MCD, like PVNH and TSC[460, 461].

Epilepsy surgery is under-utilised for TSC-associated DRE and may be successful if a dominant epileptogenic tuber is identified by EEG investigation[462]. In a large retrospective series of patients with TSC who underwent epilepsy surgery, 71% were seizure-free after 1 year and 51% were seizure-free after 10 years[377]. Surgery targeting resection of tubers and surrounding perituberal tissue was associated with better seizure outcomes compared with resections extending to the tuber margin only[377]. Patients with *TSC1* mutations had better epilepsy surgery outcomes compared to those with *TSC2* mutations, supporting the assertion that *TSC2* pathogenic variants predict a more severe neurological phenotype[377, 463].

CCMs are low-flow, haemorrhagic vascular lesions of the CNS[464]. Seizures are the presenting symptom in 25% of cases[465]. Familial CCM accounts for 20% of all cases[466]. Germline pathogenic variants in three genes, *CCM1* (*KRIT1)*, *CCM2* (*MGC4607*) or *CCM3* (*PDCD10*) were identified in 90% of cases with a positive family history[170, 467]. Familial CCM is characterised by lesions that increase in size and number over time[464]. Germline mutations in *CCM1*, *CCM2* or *CCM3* were identified in

57% of sporadic cases with multiple lesions[170, 467]. Similar to other germline genetic disorders with focal lesions, second-hit somatic mutations were detected in surgically removed CCMs[468]. Sporadic CCM cases often harbour somatic activating *PIK3CA* or *MAP3K3* mutations[171, 172].

Surgical resection or stereotactic radiosurgery are treatment options in CCM-associated DRE, particularly in cases with solitary lesions. Postoperative seizure freedom is achieved in over 75% of cases[465]. Epilepsy surgery can effectively treat multifocal CCM if a single epileptogenic focus is identified[469]. Establishing a molecular diagnosis before epilepsy surgery may assist prognostication. For example, identification of a pathogenic variant in *CCM1*, *CCM2* or *CCM3* predicts the development of *de novo* CCM. *CCM3* mutations are associated with a more severe phenotype compared with *CCM1* and *CCM2* mutations, harbouring a high rate of CCM formation (2.7 per patient per year), an increased risk of haemorrhage and unique phenotypic features including meningiomas, scoliosis and ID[470]. Somatic *PIK3CA* mutations in sporadic CCM are associated with higher risk of haemorrhage[172]. It is recommended that the siblings, children and parents of patients with *CCM1*, *CCM2* or *CCM3* mutations undergo genetic testing. MRI brain with SWI should be performed on mutation-positive relatives.

8.3.5 Somatic mosaicism in lesional epilepsies: implications for epilepsy surgery

Brain-confined somatic mutations in mTOR pathway genes are a major cause of FCD type II and HME. In large neuropathological series of surgically treated focal epilepsies, mosaic genetic variants were detected in 38% of FCD type II and HME brain specimens using targeted deep sequencing techniques[90, 91]. Mosaic *MTOR* variants accounted for two-thirds of cases with somatic mutations[90, 91]. All FCD type II specimens displayed molecular markers of mTOR hyperactivation, including those without detectable germline or somatic mutations, supporting the hypothesis that all FCD type II are mosaic mTORopathies[90]. Resective epilepsy surgery for FCD type II and HME in patients with somatic mutations in mTOR pathway genes (*MTOR*, *AKT3*, *PIK3CA*, *TSC1*, *TSC2* and *RHEB*) led to seizure freedom in 80% of cases[90, 91, 153, 155].

FCD type II has homogenous histopathologic characteristics with good epilepsy surgery outcomes in most patients[333]. Somatic mTOR gene mutations occurring in a subset of embryonic neuroglial progenitor cells give rise to the circumscribed cell proliferation abnormalities of FCD type II[142]. The site and extent of FCD type II is dependent on the timing of the somatic mutation, the targeted cell lineage and the affected gene[142].

In contrast, FCD type I is a heterogeneous group of malformations arising from late, postmigrational insults to the developing cortex[471]. In general, FCD type I is a more diffuse malformation with less favourable surgical outcomes[333]. FCD type I pathology is often found adjacent to a lesion (FCD type III), such as MTS, tumour (glial or glioneuronal) or vascular malformation. It is debated whether FCD type III is an acquired pathology with accompanying reorganisational dysplasia secondary to the principal lesion, rather than being a distinct pathologic entity. As brain MRI is often unremarkable in FCD type I, it may be difficult to demarcate the lesion margins or to establish the extent of surgical resection. FCD type I lacks specific genetic biomarkers, with rare reports of germline *SCN1A*, *KCNT1*, *PCDH19*, *CNTNAP2* and *STXBP1* mutations in patients with FCD type I[147-151].

Imaging, EEG or molecular biomarkers that predict FCD pathology could assist surgical planning and prognostication (see **Table 8.4**)[472, 473]. For example,

identification of an mTOR gene mutation supports the diagnosis of FCD type II in cases with ambiguous imaging and may predict a good surgical outcome if data is concordant. Genomic DNA methylation profiling is widely used to classify brain tumours and select targeted oncology treatments. Recent studies identified DNA methylation signatures for subtypes of FCD and other MCD, that are distinguishable using machine and deep learning methods[474, 475]. An integrated phenotype-genotype classification system has been recommended for FCD, incorporating radiological, histopathological and molecular-genetic data[476]. The proposed classification scheme could help guide treatment options, including surgical and pharmacologic therapies.

Table 8.4 MRI, EEG and genetic biomarkers of focal cortical dysplasia

	FCD type I	FCD type II
Histological findings	• Abnormal cortical lamination: radial (Ia); tangential (Ib); radial and tangential (Ic) • Cortical lamination abnormalities adjacent to hippocampal sclerosis (IIIa), tumour (IIIb), vascular malformation (IIIc) or early life acquired lesion (IIId)	• Disrupted cortical lamination with dysmorphic neurons (IIa) • Disrupted cortical lamination with dysmorphic neurons and balloon cells (IIb) • Hemimegalencephaly and cortical tubers have similar histological findings to focal cortical dysplasia type II
MRI findings	• Usually, normal • Diffuse or localised cerebral hypoplasia • Blurring of grey-white matter junction (less marked than focal cortical dysplasia type II)	• Focal cortical thickening • Increased cortical signal intensity (FLAIR and T2) • Blurring of grey-white matter junction • Focal abnormal cortical gyration • Transmantle sign
EEG signature	• Does not exhibit characteristic EEG changes	• Repetitive subcontinuous spikes, spike-and-waves, polyspikes, or paroxysmal fast activity ('brushes') interspaced with relatively flat periods
Genetic and epigenetic biomarkers	• Rare associations with germline *SCN1A*, *KCNT1*, *PCDH19*, *CNTNAP2* and *STXBP1* mutations • Potential role for DNA methylation-based profiling in differentiating FCD subtypes [474, 475]	• Somatic mutation in mTOR pathway activating genes (30-63% of cases) • Rare associations with germline mutations in mTOR suppressor genes: *DEPDC5*, *NPRL2*, *NPRL3*, *TSC1*, *TSC2* and *PTEN* • Potential role for DNA methylation-based profiling in differentiating FCD subtypes [474, 475]
Epilepsy surgery outcomes [333]	Freedom from disabling seizures • At 1 year (57.7%) • At 5 years (54.5%)	Freedom from disabling seizures • At 1 year (69.4%) • At 5 years (67.4%)

The contribution of brain somatic mosaicism to the pathogenesis of epileptogenic lesions extends beyond the mTORopathies (see **Table 8.5**). *SLC35A2* encodes a

uridine diphosphate- galactose transporter that carries galactose into Golgi vesicles for glycosylation. Germline *SLC35A2* mutations cause a rare X-linked dominant form of DEE[477]. Brain somatic mutations in *SLC35A2* were detected in patients with lesional DRE characterised on MRI by corticomedullary junction T2 high signal in young children and reduced corticomedullary differentiation in older patients due to abnormal hyperintensity of adjacent white matter[159]. Neuropathological findings were consistent with MOGHE in most cases[159]. Low-level somatic mutations in *SLC35A2* were also identified in three patients with non-lesional focal epilepsy, of whom two had pathological findings of FCD type Ia[152]. Epilepsy surgery led to seizure freedom in 71% of cases after a mean follow-up of 2.5 years[152, 159].

Long-term epilepsy-associated tumours (for example, ganglioglioma and DNET) are common causes of DRE in young patients. Ganglioglioma was the second most common diagnosis in a large neuropathological series of surgically treated epilepsies[478]. Gangliogliomas are slow-growing glioneuronal tumours, associated with DRE in ~90% of cases. Tumour resection led to seizure freedom in over 90% of cases[479]. The somatic *BRAF* V600E (NM_004333.4:c.1799T>A:p.Val600Glu) mutation was detected in 58% of brain tissue specimens in a large series of gangliogliomas[480]. *BRAF* V600E mutations in paediatric gangliogliomas are associated with an increased risk of tumour recurrence[160]. In a mouse model of ganglioglioma, *Braf* mutation in neural progenitor cells resulted in dysplastic epileptogenic neurons, while mutated glial cells lacked epileptogenicity. A BRAF inhibitor (vemurafenib) developed for the treatment of advanced melanoma reduced seizures in mutant mice. These findings suggest that the intrinsic epileptogenicity of gangliogliomas is produced by somatic *BRAF* mutation in tumour neurons[481]. In a similar fashion, mutations in *IDH1* contribute to the epileptogenicity of gliomas[482].

Table 8.5 Somatic mosaicism in focal epilepsies with structural causes

Mutated gene(s)	Lesion(s)
SLC35A2	Mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy Focal cortical dysplasia type I
MTOR, AKT3, PIK3CA, *PIK3R2, RHEB*, *TSC1, TSC2, PTEN*	Focal cortical dysplasia type II Bottom-of-sulcus dysplasia Hemimegalencephaly Polymicrogyria
BRAF p.Val600Glu	Ganglioglioma
FGFR1	Dysembryoplastic neuroepithelial tumour
GNAQ	Leptomeningeal angiomatosis (Sturge Weber syndrome)
PRKACA, GL13 (Genes involved in regulation of the sonic hedgehog pathway)	Hypothalamic Hamartoma
DCX, LIS1	Lissencephaly spectrum malformations
FLNA	Periventricular nodular heterotopia
PIK3CA, MAP3K3	Cerebral cavernous malformations

A mutational gradient was demonstrated in FCD specimens harbouring somatic mutations in *MTOR*, *DEPDC5, RHEB* and *SLC35A2*[90, 143, 153, 158, 159, 244]. The mosaic

gradient was evident in cases where multiple brain tissue specimens were resected. VAFs were highest in brain regions with severe electrophysiologic findings and increased concentrations of dysplastic cells. Mutational loads were lower in the surrounding epileptogenic border and absent from adjacent normal tissue[143, 244]. Focused resection or ablation of brain tissue with high levels of somatic mosaicism may improve surgical outcomes, while subtotal resection of mutated tissue may predict seizure recurrence.

Specific somatic *MTOR* mutations (NM_004958.3:c.6644C>T:p.Ser2215Phe) may induce strong mTOR pathway activation in FCD tissue, even in cases with low-level mosaicism[483]. Two patients underwent complete hemispherectomy for FCD type II due to hyperactivating *MTOR* mutations. Both unexpectedly developed postoperative seizures arising from the contralateral, residual hemisphere. *MTOR* mutations with strong hyperactivating properties may increase seizure recurrence risk due to bilateral asymmetric hemispheric abnormalities[483]. Moreover, the occurrence of two somatic variants affecting different activating genes within the mTOR pathway appeared to synergistically effect the phenotype of a child with left HME[484]. Complete left hemispherectomy failed to control seizures, with postoperative seizures arising from the apparently normal right hemisphere. The patient had a brain somatic *MTOR* mutation (~8.8% mosaicism in brain) and a systemic mosaic *RPS6* mutation (~15.1% mosaicism in brain, 11% mosaicism in blood). A double mutant animal model exhibited more severe neuropathological findings compared with models overexpressing the two variants independently[484].

At present, brain tissue is prerequisite to reliably detect somatic mutations in patients with MCD. Cell-free DNA are short fragments of non-encapsulated DNA released into the bloodstream or cerebrospinal fluid (CSF). Liquid biopsy of cell-free DNA has established clinical applications in cancer diagnostics and disease monitoring, including CSF liquid biopsy for detection of somatic mutations in malignant brain tumours[485]. Applying CSF liquid biopsy as a surrogate to detect somatic mosaicism in epilepsy associated MCD may obviate the need for brain tissue[486, 487]. In a study of 12 epilepsy surgery patients with somatic mutations detected in brain tissue, one-quarter had the same somatic mutation identified in CSF-derived cell-free DNA obtained by dural puncture (*PIK3CA* variant in HME case, *BRAF* variant in ganglioglioma case and

SLC35A2 variant in MOGHE case)[486]. The mosaic variants were not identified in lymphocyte-derived DNA from blood samples. Establishing a genetic diagnosis in epilepsy associated MCD prior to surgery would improve prognostication and facilitate early adoption of potential precision therapies. The presence of mutated brain DNA in CSF obtained after surgery might be predictive of seizure relapse, although this hypothesis warrants further investigation.

8.4 Discussion

Currently, patients referred for epilepsy surgery undergo an extensive presurgical evaluation incorporating vEEG, high-field MRI, metabolic imaging and in a growing number of patients, intracranial EEG. Increasingly complex cases are now considered surgical candidates due to SEEG, particularly patients with extra-temporal epilepsies and/or normal MRI. As the complexity of surgical epilepsy increases, so too must our efforts to provide accurate molecular diagnosis in DRE. Genetic testing is not yet routinely included in the presurgical evaluation, despite the reduced cost and wider availability of NGS in clinical practice.

Although limited by small numbers, evidence supports that genetic testing in focal epilepsies may inform predictions about surgical candidacy and outcomes. The greatest value is in the presurgical evaluation of 'MRI negative' focal epilepsy. Approximately two-thirds of patients with DRE caused by mutations in mTOR pathway genes (predominantly GATOR1-related epilepsies) experienced favourable epilepsy surgery outcomes. Five out of eight patients with 'MRI negative' GATOR1-related epilepsies achieved Engel Class I or II surgical outcomes[127, 421, 423]. Finding a germline GATOR1 mutation in 'MRI negative' focal epilepsy suggests an underlying microstructural lesion and supports further investigation with intracranial EEG, particularly if seizure semiology and vEEG suggest a single focus.

In contrast, mutations in ion channel and synaptic transmission genes suggest a more extensive epileptogenic network or dysplasia, with less favourable surgical outcomes in most cases. In such cases, if EEG reveals generalised or multifocal discharges in addition to focal discharges, resection may not be indicated unless seizures consistently arise from a single focus. However, genomic data should not be used in isolation to preclude epilepsy surgery and should be considered alongside other diagnostic modalities. For example, a subset of patients with *SCN1A*-related mesial temporal lobe epilepsy and concordant MTS benefited from surgery[426]. The role of neuromodulation in channelopathies and synaptopathies warrants further study.

In patients with 'MRI positive' focal epilepsies, genetic studies may uncover an underlying diathesis, facilitating more accurate aetiologic classification and in some cases, may indicate epileptogenic potential beyond the extent of the visible lesion. Presurgical germline genetic testing is recommended in patients with FCD to screen

for mutations that may confer increased risk of postoperative seizure relapse, as with *SCN1A* and *KCNT1* mutations[147, 148, 427]. However, these observations originate from few cases and no studies have rigorously addressed whether certain mutations contraindicate epilepsy surgery. It is now widely established that somatic mosaicism plays a significant role in the development of lesional focal epilepsies. Postoperative genetic analysis of brain tissue may provide useful prognostic information, or in rare cases explain surgical failure[483, 484]. Less invasive diagnostic methods that can detect brain somatic mutations without brain tissue are currently under development.

Neurologists should consider genetic testing in focal non-lesional DRE, epilepsy in the setting of ID and epilepsy with focal MCD. Comprehensive genetic investigation utilising WES or WGS is recommended in focal non-lesional epilepsies and DEE, while single gene analysis or targeted panels can be employed in structural epilepsies like TSC and familial CCM. Accurate variant interpretation is essential and requires the expertise of an epilepsy genetics MDT. It is anticipated that genetic testing will become routine practice in the presurgical evaluation, and that integration of genomic data with other diagnostic modalities will improve surgical outcomes. A recent survey of German epilepsy specialists revealed that 88% of participants approved the incorporation of genetic testing into the presurgical evaluation[422].

Including genomic data in the epilepsy surgery evaluation fosters a personalised therapeutic approach. Precision therapies using small molecules or gene therapies may become alternative to epilepsy surgery in some genetically mediated epilepsies. Everolimus could be trialled in TSC-related epilepsy or other mTORopathies if surgery is not feasible or as a bridging therapy, if intracranial EEG is planned[136]. Potential gene therapy strategies include CRISPR-Cas9 gene editing, as used in a mouse model of Dravet syndrome to upregulate interneuron *Scn1a* expression[193], or ASOs that inhibit mRNA translation, as utilised in a mouse model of GoF *SCN8A*-related DEE to reduce *Scn8a* transcripts[488]. Gene therapies were delivered via intracerebral injection in both mouse models. Successful clinical translation will require a collaborative MDT approach for patient selection and treatment delivery, particularly if gene therapies require neurosurgical administration.

Reports on the surgical outcomes of patients with monogenic epilepsies were largely drawn from small case series and case reports. Consequently, the findings from the systematic review may be subject to publication bias, that is, a tendency for studies with positive results to be published. Large multicentre prospective studies are needed to determine if genetic testing in the presurgical evaluation yields improved surgical outcomes.

In conclusion, we advocate for including genomic data in the presurgical evaluation and multidisciplinary discussion of many epilepsy surgery candidates. The integration of genomics into the presurgical evaluation assists selection of patients for resective surgery and fosters a personalised medicine approach, where precision and targeted therapies are considered alongside surgical procedures.

9. General Discussion

9.1 Addressing the thesis objectives

The primary objective of this thesis is to describe and evaluate the application of precision medicine to the management of epilepsy in mTORopathies, with a specific focus on GATOR1-related epilepsies. During my clinical training I frequently encountered patients with severe epilepsies who responded poorly to conventional treatment approaches. Recent advancements in genomic diagnostics have demonstrated that many severe epilepsies have a genetic basis. This prompted me to consider the potential of treatments that target underlying disease biology to improve outcomes in monogenic epilepsies. The central aim of precision medicine is to leverage genetic and molecular data to inform clinical decision-making and optimise therapies.

In chapter 3, I described the clinical and molecular features of mTORopathies, a group of rare genetic disorders characterised by refractory epilepsy, MCD and neuropsychiatric symptoms. To date, pathogenic variants in 17 genes encoding components of the mTOR pathway have been found to cause epilepsy and neurodevelopmental disorders. Since mTORC1 hyperactivation has been linked to epileptogenesis in all mTORopathies, rapalogues may offer a more targeted treatment strategy compared to traditional ASMs. In chapter 4, the clinical evidence supporting mTOR inhibitor treatment for epilepsy in mTORopathies was reviewed. Class I evidence supports the safety and efficacy of mTOR inhibitors in TSC. However, clinical evidence supporting the use of mTOR inhibitors in non-TSC mTORopathies is largely derived from small case series and case reports, which are prone to publication bias and may not accurately reflect their true effectiveness.

Chapter 5 presented the Irish experience of using everolimus for DRE in TSC. Prior to the study, everolimus was rarely prescribed in Irish clinics for refractory epilepsy in TSC, despite strong evidence supporting its efficacy. Following efforts to increase awareness and establish dosing and monitoring protocols, the percentage of eligible TSC patients treated with everolimus increased from 13 to 42%. Of the 13 TSC patients treated with everolimus, seven had significant seizure frequency reductions, including one patient who became seizure-free before stopping treatment to become pregnant. Most patients experienced side-effects, with stomatitis and

hypercholesterolemia being the most frequent. Optimal TSC management involves a multidisciplinary TSC clinic with a dedicated nurse specialist to oversee mTOR inhibitor treatment and radiological surveillance of multisystem manifestations. Telemedicine could be utilised to integrate multidisciplinary expertise.

A phenotyping analysis of Irish patients with GATOR1-related epilepsies was presented in Chapter 6. GATOR1-related epilepsies were found to be the third most common cause of monogenic epilepsy amongst Beaumont Hospital patients, after TSC and *SCN1A*-related epilepsies. The analysis estimated that GATOR1-related epilepsies comprise less than 0.2% of the overall epilepsy population attending Beaumont Hospital. Clinical and genetic characteristics of nine patients with GATOR1-related epilepsies were analysed, including three sisters. All patients were identified through genomic research programmes at the FutureNeuro Research Centre, and CHI at Temple Street. Five patients had DRE, with a median baseline seizure frequency of 18 per month. The high rates of refractory epilepsy and neuropsychiatric co-morbidities align with the findings of Baldassari *et al*[127]. Novel observations included the diagnosis of PNES through vEEG in two patients with GATOR1-related epilepsies, and the detection of ictal asystole during vEEG monitoring in a man with *DEPDC5*-related epilepsy. Ictal asystole may contribute to the increased risk of SUDEP in some patients with GATOR1-related epilepsies, although Bacq and colleagues did not identify cardiac abnormalities in *Depdc5* mouse models and patients with *DEPDC5* mutations[411].

In Chapter 7, a pilot study on the use of everolimus as a precision treatment for refractory GATOR1-related epilepsies was presented. The study involved five patients who received everolimus for a median duration of 12 months (range 7-31 months). The three patients with *DEPDC5* LoF variants experienced significant seizure frequency reductions (74.3- 86.1%). A patient with a *DEPDC5* missense variant experienced a 40% reduction in seizures, although focal to bilateral tonic-clonic seizures remained active. The patient with *NPRL3*-related epilepsy did not show any response to everolimus treatment. Stomatitis was common but manageable with conservative measures.

Chapter 8 presented a literature review on the responsiveness of monogenic epilepsies to epilepsy surgery. Focal epilepsies caused by germline and somatic variants in mTOR pathway genes are often amenable to epilepsy surgery, whereas epilepsies caused by variants in ion channel and synaptic transmission genes tend to have less favourable surgical outcomes. The integration of genomics into the presurgical evaluation can assist in selecting suitable patients for epilepsy surgery, and fosters a precision medicine approach, where targeted therapies are considered alongside surgical procedures[117].

The next section presents recommendations for epilepsy management in mTORopathies using a precision medicine approach. These recommendations are based on the findings from the literature reviews and everolimus studies discussed in previous chapters.

9.2 Precision medicine approaches in mTORopathies: evidence-based recommendations for epilepsy management

9.2.1 Tuberous sclerosis complex

Infants displaying clinical features of TSC should undergo early genetic testing for pathogenic variants in *TSC1* or *TSC2*. Patients with *TSC2* mutations should be prioritised for early EEG monitoring, as *TSC2* mutations are associated with more severe neurological phenotypes, including infantile spasms[373]. Serial EEG monitoring is recommended in pre-symptomatic TSC, as prophylactic administration of vigabatrin at the onset of epileptiform abnormalities has been shown to improve long-term epilepsy and cognitive outcomes[391]. There are several evidence-based treatment options for refractory epilepsy in TSC (see **Figure 9.1**), including everolimus[135], CBD[400], and epilepsy surgery with resection of tubers and surrounding perituberal tissue[377]. Early treatment with everolimus in seizure naïve TSC patients could lead to additional long-term epilepsy and cognitive benefits, although further research is needed to confirm this hypothesis, ideally through prospective longitudinal analysis. Additional research is required to determine the safety of everolimus in children younger than two years.

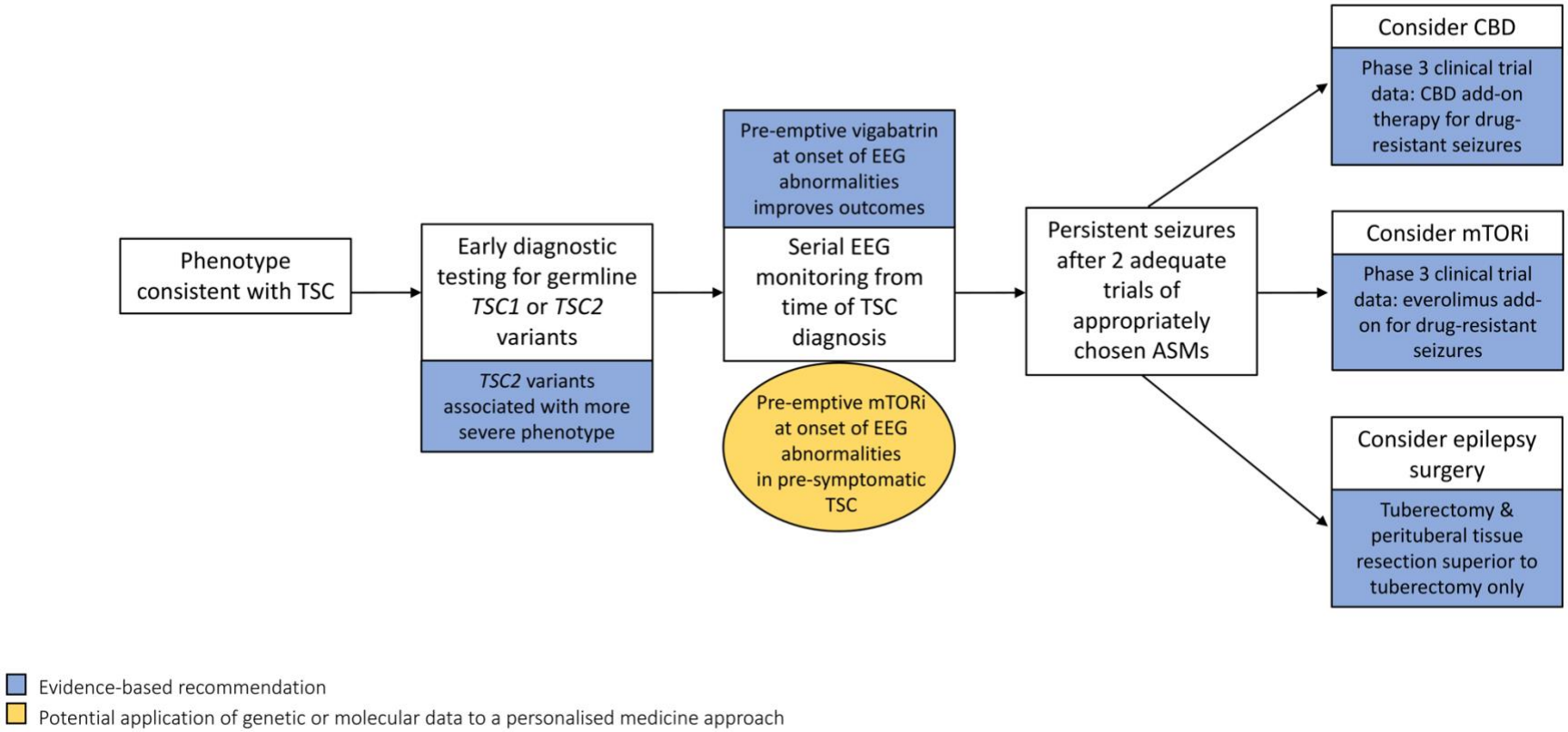

Figure 9.1 Precision medicine approach for managing epilepsy in tuberous sclerosis complex

Figure taken from Moloney, Cavalleri and Delanty, 2021 [136]

Abbreviations:
CBD= cannabidiol; mTORi= mechanistic target of rapamycin inhibitor.

9.2.2 GATOR1-related epilepsies

Patients with non-lesional focal DRE should undergo diagnostic exome sequencing for pathogenic variants in *DEPDC5, NPRL2* and *NPRL3*. Exome sequencing should also be considered in patients with refractory epilepsy due to FCD type II or HME. LoF variants in GATOR1 genes are associated with severe epilepsy phenotypes, including FCD, epileptic spasms and SUDEP[127]. Treatment options for refractory GATOR1-related epilepsies include epilepsy surgery, and potentially mTOR inhibitors (see **Figure 9.2**). Favourable epilepsy surgery outcomes were observed in over 60% of patients with GATOR1-related epilepsies[117]. Our preliminary data suggests that everolimus is a promising treatment option for refractory GATOR1-related epilepsies, particularly those caused by LoF variants in *DEPDC5.*

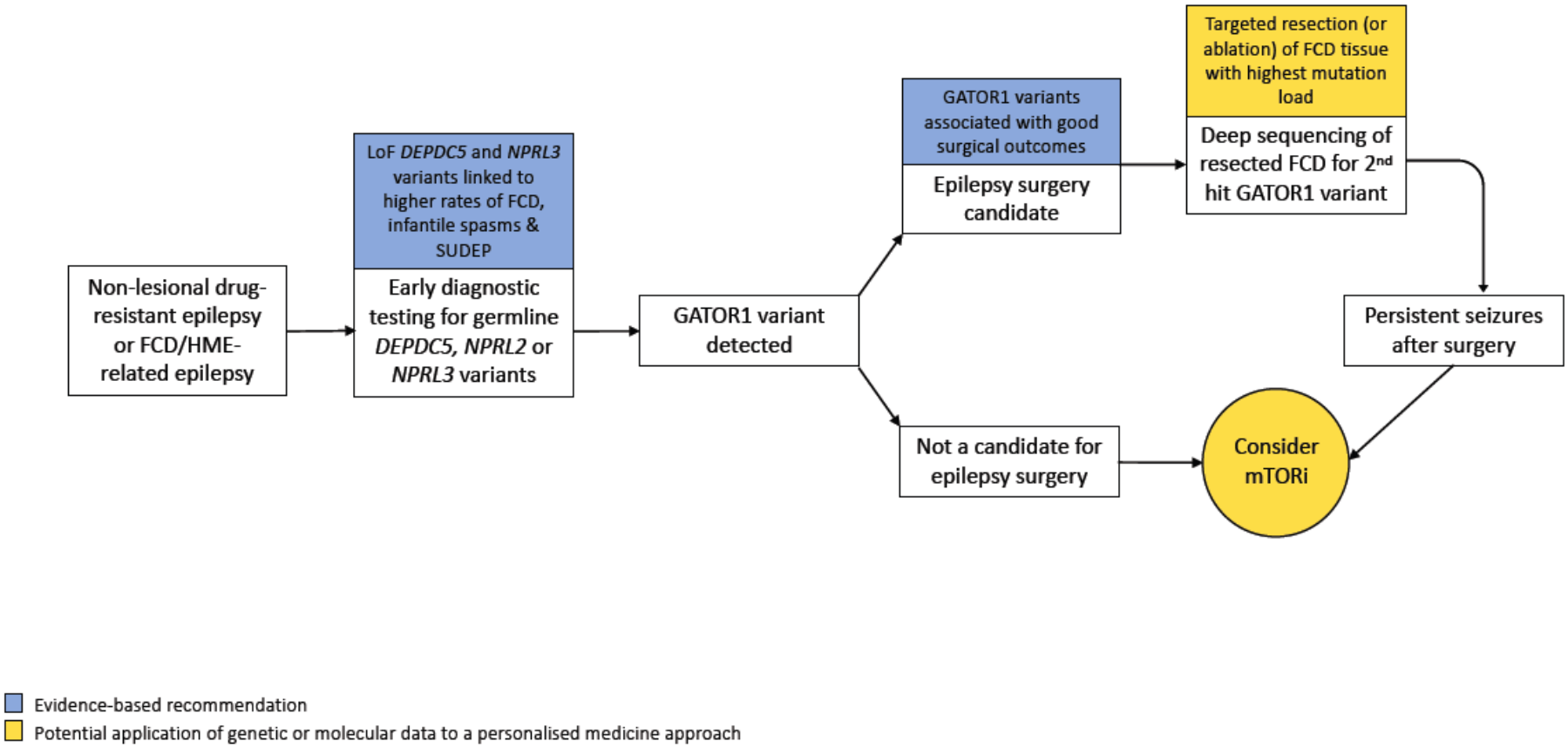

Figure 9.2 Precision medicine approach for managing GATOR1-related epilepsies

Figure taken from Moloney, Cavalleri and Delanty, 2021 [136]

Abbreviations:
FCD= focal cortical dysplasia; HME= hemimegalencephaly; LoF= loss-of-function; mTORi= mechanistic target of rapamycin inhibitor; SUDEP= sudden unexpected death in epilepsy.

9.2.3 Focal cortical dysplasia type II and hemimegalencephaly

Resective epilepsy surgery for FCD type II and HME leads to good seizure outcomes in most appropriately selected patients. Presurgical genetic testing is recommended in patients with FCD and HME. Finding a germline variant in an mTOR pathway gene supports the diagnosis of FCD type II in cases with ambiguous imaging and may predict a good surgical outcome in cases with concordant EEG and MRI data[117]. Conversely, certain mutations may confer increased risk of postoperative seizure relapse, such as variants in *SCN1A* or *KCNT1*[147, 148, 427].

Treatment with everolimus has the potential to improve seizure control in patients with surgically inaccessible FCD, or patients who continue to experience seizures after epilepsy surgery. Alpelisib, a selective PIK3CA inhibitor, improved clinical outcomes in patients with *PIK3CA*-related overgrowth syndromes[489]. Somatic *PIK3CA* variants also cause FCD type II and HME[90, 91]. A child with HME in the context of *PIK3CA*-related overgrowth syndrome had improved seizure control on targeted treatment with the AKT inhibitor, miransertib[490]. PIK3CA and AKT inhibitors are potential targeted treatment options for FCD caused by somatic GoF mutations in *PIK3CA* and *AKT3* (see **Figure 9.3**).

Postoperative genetic analysis of brain tissue can provide valuable prognostic information, and in rare cases may explain surgical failure. Specific somatic variants in *MTOR* (for example, c.6644C>T, p.Ser2215Phe) are associated with strong activation of the mTOR pathway, and may increase seizure recurrence risk due to bilateral asymmetric hemispheric abnormalities[483]. Current research aims to develop less invasive diagnostic methods, such as CSF liquid biopsy of cell-free DNA. This method has the potential to detect brain somatic mutations without the need for brain tissue[486].

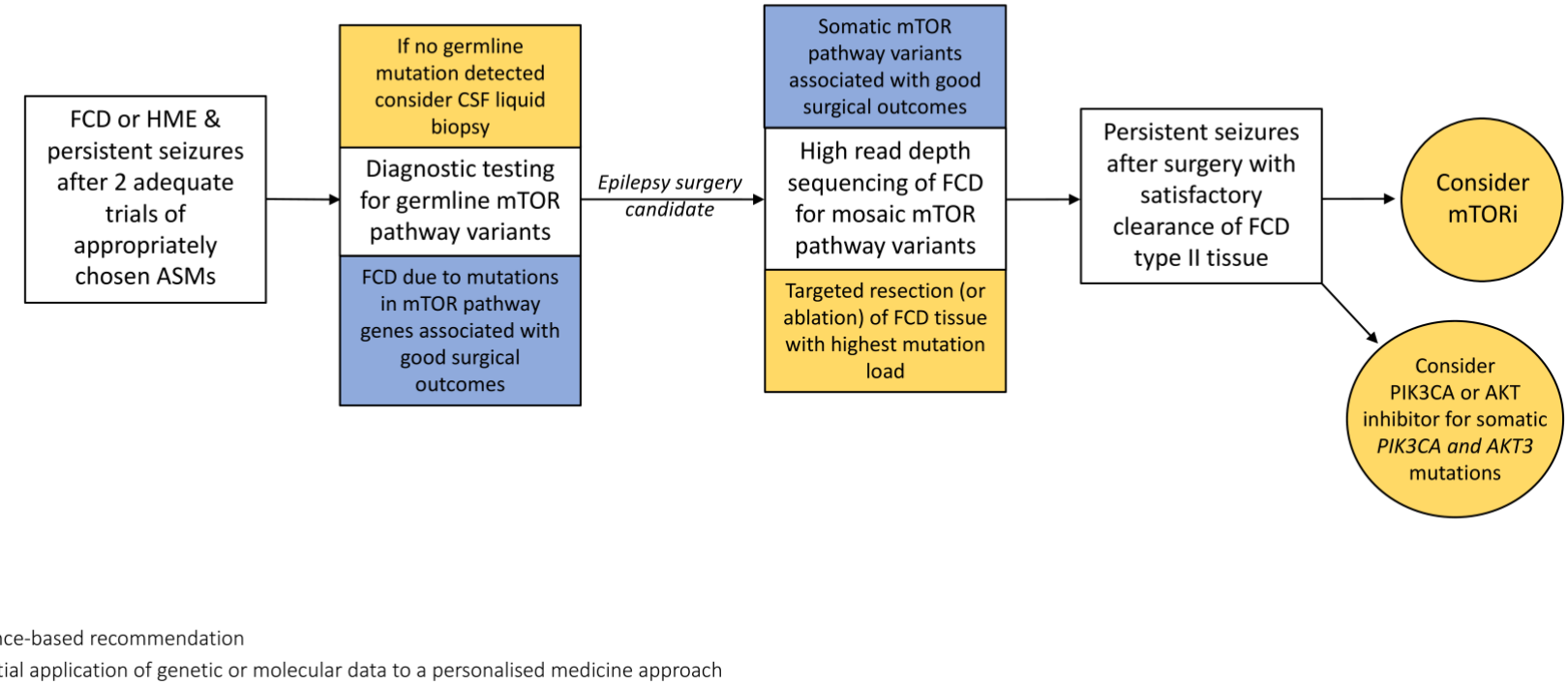

Figure 9.3 Precision medicine approach for managing refractory epilepsy caused by FCD type II and hemimegalencephaly

Figure taken from Moloney, Cavalleri and Delanty, 2021 [136]

Abbreviations:
HME= hemimegalencephaly; mTORi= mechanistic target of rapamycin inhibitor.

9.3 Precision medicine limitations in mTORopathies and other monogenic epilepsies

Findings from this thesis demonstrate that everolimus precision treatment can improve seizure control in some patients with GATOR1-related epilepsies and TSC. However, a significant proportion of patients derived no clinical benefit from everolimus. Similarly, 60% of TSC patients in the EXIST-3 trial did not respond to everolimus treatment[135]. In the present study, it was not possible to identify reliable predictors of treatment response due to the small sample size. Patients with LoF variants in *DEPDC5* had the most substantial seizure frequency reductions, though further studies with larger numbers are needed to validate this finding. GATOR1-related epilepsies and TSC are characterised by phenotypic heterogeneity, which may partially explain the diverse treatment responses. Second-hit somatic variants likely contribute to phenotypic variability in mTORopathies. Epigenetic and environmental factors may also influence disease expression.

The extreme chronicity and intractability of epilepsy in the cohort might explain treatment failure in some patients. The concept that "*seizures beget seizures*," or that recurrent seizures and frequent interictal epileptiform discharges can lead to increasingly severe, treatment-resistant epilepsy, was first proposed by Sir William Gowers in 1881[491]. His theory is supported by clinical and experimental evidence from mesial temporal lobe epilepsies and DEE[492]. However, evidence for seizure-dependent worsening in other forms of epilepsy is lacking[492]. Progressive structural and EEG abnormalities are well described in TSC-related epilepsies[389, 390], suggesting that the timing of intervention may affect outcomes in TSC, and potentially other mTORopathies. Indeed, treatment with pre-emptive vigabatrin delayed the onset of epilepsy, and reduced the severity of seizures and cognitive impairment in seizure naïve infants with TSC[391]. Additional benefits may be achievable with early everolimus treatment. Pathogenic sequence changes in genes involved in brain development, including mTOR pathway genes, may activate epileptogenesis through developmental alterations in brain structure. These structural changes may not be readily reversible with precision therapies.

Tolerability issues and pharmacokinetic constraints may also contribute to suboptimal treatment response in certain patients. Dose-dependent stomatitis limited attainment of target serum everolimus levels in some patients. Limited penetration of everolimus across the blood-brain-barrier might explain reduced efficacy in some patients. ATP-competitive mTOR kinase inhibitors with enhanced CNS penetration and reduced systemic side-effects are being developed, and have shown promise as seizure treatments in mouse models of TSC[365].

All currently available precision therapies for monogenic epilepsies, including everolimus, fall within Tier 3 of Byrne and colleagues' hierarchy of increasing therapeutic precision[179]. Tier 3 treatments modulate or bypass the dysfunction caused by the mutated gene product, which in the case of mTORopathies is mTORC1 hyperactivation. However, Tier 3 treatments do not restore normal gene function, which likely limits their efficacy in severe neurological disorders. More substantial clinical improvements are expected with precision treatments that modulate gene expression by targeting RNA (i.e, ASO therapies), and treatments that replace the mutated gene with a normal functioning copy (i.e, gene replacement therapy) [179]. Currently, ASOs and gene replacement therapies are not available for genetic epilepsies.

Despite these limitations, everolimus represents a viable treatment option for DRE in TSC, GATOR1-related epilepsies, and potentially other mTORopathies. Everolimus targeted therapy should be considered in conjunction with other high efficacy treatments, like epilepsy surgery, neuromodulation and recently approved ASMs, such as cenobamate for refractory focal epilepsy, and CBD for TSC. Findings from the epilepsy surgery literature review indicate that mTORopathies are amenable to resective surgeries, when seizure semiology, EEG and imaging data are concordant[117]. Treatment selection strategies for patients with monogenic epilepsies should employ principles from stratified and personalised medicine. '*High-definition*' medicine has been proposed as a desirable extension to the current direction of precision medicine in epilepsy care[1]. A high-definition approach involves "*dynamic assessment, management, and understanding of an individual's health measured at (or near) its most basic units*"[493]. The objective of high-definition medicine is to target multiple factors that influence outcomes, including underlying molecular-biological

pathways, timing of intervention, polygenic risk, pharmacogenomics, microbiomics and modifiable lifestyle factors[1]. To be successful, this data-driven approach will require sophisticated analytical techniques that harness the potential of artificial intelligence.

Precision medicine is criticised for its narrow applicability to a limited number of patients. The vast majority of monogenic epilepsies are extremely rare, with TSC and Dravet syndrome being notable exceptions. Despite the promise of precision medicine, there remains a dearth of robust, large-scale evidence supporting the use of precision therapies for monogenic epilepsies, with the exception of everolimus for TSC-related epilepsy. Parallel group randomised controlled trials may not be feasible in rare mTORopathies, like the GATOR1-related epilepsies. '*N-of-1*' trials are recommended for evaluating treatments in rare genetic disorders with episodic symptoms, such as seizures. N-of-1 studies are randomized, controlled, multiple crossover trials in single patients, allowing the participant to act as their own control (see **Figure 9.4**)[196].

A recently published n-of-1 trial evaluated the efficacy of inhaled salbutamol as an acute treatment for hyperkalaemic periodic paralysis in a patient with a pathogenic variant in *SCN4A*[494]. Salbutamol is a rational choice to treat this condition, as it promotes cellular uptake of potassium. The patient was blinded to treatment for acute attacks. Twenty-three attacks were treated with salbutamol, and 22 with aerosolised placebo. No significant differences were observed between attacks treated with salbutamol or placebo. The patient agreed to discontinue salbutamol after being informed of the results. Prophylactic acetazolamide was introduced, which led to reduced attack frequency[494]. The study illustrates the value of conducting n-of-1 trials in routine clinical practice to inform and optimise individual patient management. The n-of-1 trial design offers a methodologically rigorous approach for providing evidence-based, personalised medicine. Pooled data from multiple n-of-1 trials can produce robust estimates of treatment outcomes[196].

Practical challenges curtail more widespread clinical application of n-of-1 trials. A trial pharmacist is needed to prepare active and placebo treatments, to handle randomisation procedures, and to ensure blinding. Trial design and statistical analyses

are often complex, requiring medical statistician support. These endeavours are costly and funders may be reluctant to finance single-patient trials due to concerns about lack of generalisability. Most importantly, n-of-1 trials place significant time and commitment demands on physicians and patients, compared to simply prescribing a medication[196].

The establishment of clinical registers of solved genetic epilepsies would facilitate more efficient identification of suitable patients for precision medicine trials. Effective governance, privacy protection, secure data storage and curation are essential to successful implementation of clinical registers[1]. Professor Delanty and Professor Cavalleri have led efforts to establish an epilepsy-associated gene ready (EAGER) database, collaborating with international partners from New York, Toronto, Sydney, Melbourne, Oxford, Brussels and Newcastle. Patients and families will consent to be contacted about gene-specific clinical trials across participating sites. '*N-of-some*' trials involving well organised clinical networks of patients with mutations in the same gene could help facilitate precision medicine trials.

Off-label drug prescribing may be justified in severe monogenic epilepsies, particularly with repurposed drugs that target disease-specific molecular mechanisms. For example, two patients with *SCN8A*-related DEE experienced significant seizure frequency reductions on off-label riluzole treatment. The decision to initiate riluzole was based on evidence from in *vitro* electrophysiological experiments, its favourable safety profile in amyotrophic lateral sclerosis, and the severity of the patients' epilepsy[195]. Similarly, off-label treatment with everolimus may be clinically justified in severe GATOR1-related epilepsies, supported by reports of rapamycin-responsive seizures in GATORopathy mouse models[262, 263], the safety profile of everolimus in TSC, and findings from our pilot observational study. Explicit consent is prerequisite when off-label treatments are used experimentally, or as part of research. Obtaining informed consent from patients with severe mTORopathies may be challenging, as many have co-morbid ID. In certain patients lacking decision-making capacity, starting off-label treatment with consent from a family member or caregiver may be in their best interest. Multidisciplinary decision-making regarding repurposed treatments or novel precision therapies for rare genetic disorders will help facilitate appropriate patient selection for specific interventions.

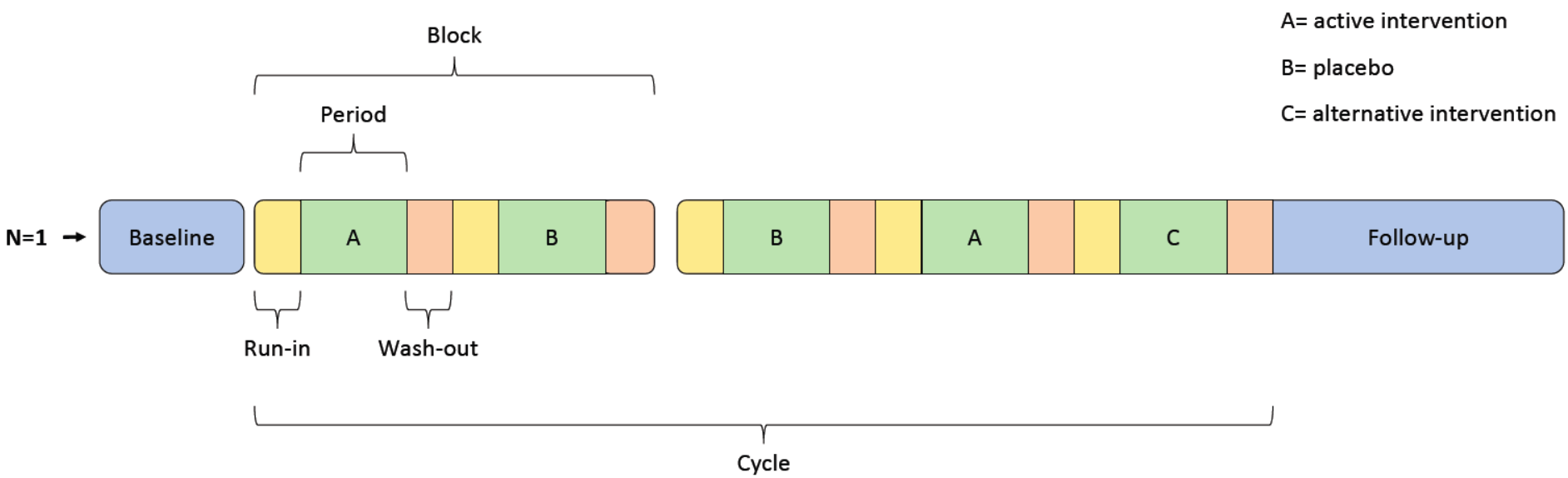

Figure 9.4 Schematic representation of the n-of-1 trial design

Figure adapted from Müller et al, 2021 [495].

Definitions:

Run-in= time preceding starting treatment at intended dose to avoid sudden introduction of a fixed therapeutic dose.

Period= duration of an intervention or comparator.

Block= a repeated unit of a set number of periods.

Cycle= each repeated unit of a set number of periods within a sequence.

Wash-out= time without an intervention following a treatment period to ensure that effects of treatment have disappeared

9.4 Future Directions

Several unresolved issues emerged during the course of the MD project. I aim to address these through future research and health service development. These issues are outlined in bullet points below.

- Current data on the efficacy and tolerability of everolimus treatment for GATOR1-related epilepsies is preliminary. A larger, multicentre study of everolimus for refractory GATOR1-related epilepsies would provide a more robust evaluation. Eligible patients could be recruited from gene-ready clinical registers. Alternatively, pooled data from multiple well-designed n-of-1 trials could also provide strong evidence. Our findings on the efficacy of everolimus in GATOR1-related epilepsies have been shared with a research group at the University Medical Centre, Utrecht, The Netherlands. This research, led by Dr. Victoria Defelippe and Dr. Floor Jansen, aims to design and conduct n-of-1 trials for patients with GATOR1-releated epilepsies.

- The clinical spectrum of mTORopathies, as outlined in Chapter 3, encompasses both common (for example, FCD type II) and rare (for example, *SZT2*-related DEE) disorders, all marked by a propensity towards refractory epilepsy. These disorders share neuropathological hallmarks, including disorganised cortical lamination, cytomegalic neurons, neuronal hyperexcitability, and dysregulated mTORC1 signalling. Given this shared neurobiology, it is plausible that mTOR inhibitors could be effective in treating epilepsy in all mTORopathies. FCD type II, the most common mTORopathy, is a major cause of childhood-onset DRE. A small open-label prospective study found that sirolimus provided modest benefit in patients with persistent seizures after epilepsy surgery for FCD type II[334]. To provide a more robust evaluation of mTOR inhibitor treatment for FCD type II, future research should employ a parallel group design with an extended follow-up period, as well as more comprehensive pathological and genomic characterisation of FCD. N-of-1 trials, or “n-of-some” trials involving well-organised clinical networks of patients

with mutations in the same gene could be used to study mTOR inhibitor treatment in rare mTORopathies.

- The healthcare needs of patients with TSC are best managed in specialised multidisciplinary TSC clinics. There is no specialised TSC clinic in Ireland. The multisystem manifestations of TSC require clinical input from neurologists, nephrologists, respiratory specialists, cardiologists, neurosurgeons, dermatologists and ophthalmologists, along with thorough radiological surveillance. General neurologists lack experience using mTOR inhibitors. In the future, I aim to establish a TSC clinic in Ireland, with access to specialised, multidisciplinary services, and telemedicine-based MDT meetings to facilitate discussion about complex cases.

- Epilepsy surgery remains the most efficacious treatment option for focal DRE. The presurgical epilepsy evaluation aims to identify suitable candidates, establish surgical plans, and forecast outcomes. Findings from the literature review suggest that appropriate use of genomic biomarkers can improve patient selection for epilepsy surgery and assist predictions on surgical outcomes. Large multicentre studies of epilepsy surgery outcomes in patients with monogenic epilepsies are needed to determine whether genetic testing in the presurgical evaluation translates to improved surgical outcomes. Seizure outcome data was gathered for all patients with monogenic epilepsies who underwent epilepsy surgery or VNS insertion at Beaumont Hospital and will be analysed as part of an international multicentre study.

9.5 Final reflections on this MD project

During the course of this MD project, I developed a diverse range of transferable skills, including proficiency in systematic literature review, genomic analysis and clinical research. The field of epilepsy genetics is rapidly advancing, and the specific skills that I have acquired in genomic analysis and interpretation will be highly valuable in both clinical and research settings in the future. The academic relationships that I have established with the FutureNeuro research centre, RCSI, and CHI at Temple Street will hopefully endure and continue to be fruitful. My short-term goal is to expand my knowledge and skills in genomic analysis and interpretation, as well as management of patients with complex neurogenetic disorders. I was awarded the Dr. Richard Steevens scholarship, which has enabled me to undertake a clinical fellowship in adult neurogenetics at the National Hospital of Neurology and Neurosurgery in London. My long-term aspiration is to lead and develop a fully integrated neurogenetics unit in Ireland, providing expertise in clinical assessment, molecular diagnostics, genetic counselling, longitudinal care, and precision therapeutics.

The research project's most impactful contribution is likely to be the study of everolimus precision treatment for GATOR1-related epilepsies. This study is the first to present clinical data on the potential benefit of add-on everolimus treatment for *DEPDC5*-related epilepsies. The successful execution of this project was made possible through collaboration between translational scientists and clinicians. Genetic diagnoses were established via genomic diagnostic research and discussion involving our epilepsy genetic MDT. Eligible patients were treated with a repurposed drug directed towards the underlying disease biology (see **Figure 9.5**). This "*n-of-some*" trial design could serve as a model for future precision medicine trials in epilepsy. Many questions in relation to precision medicine may not be answerable through traditional parallel group trials. Instead, high quality prospectively gathered data, if refined and scaled up, may provide more insight. Randomised controlled trials may be useful for more prevalent genetic epilepsies, such as Dravet syndrome.

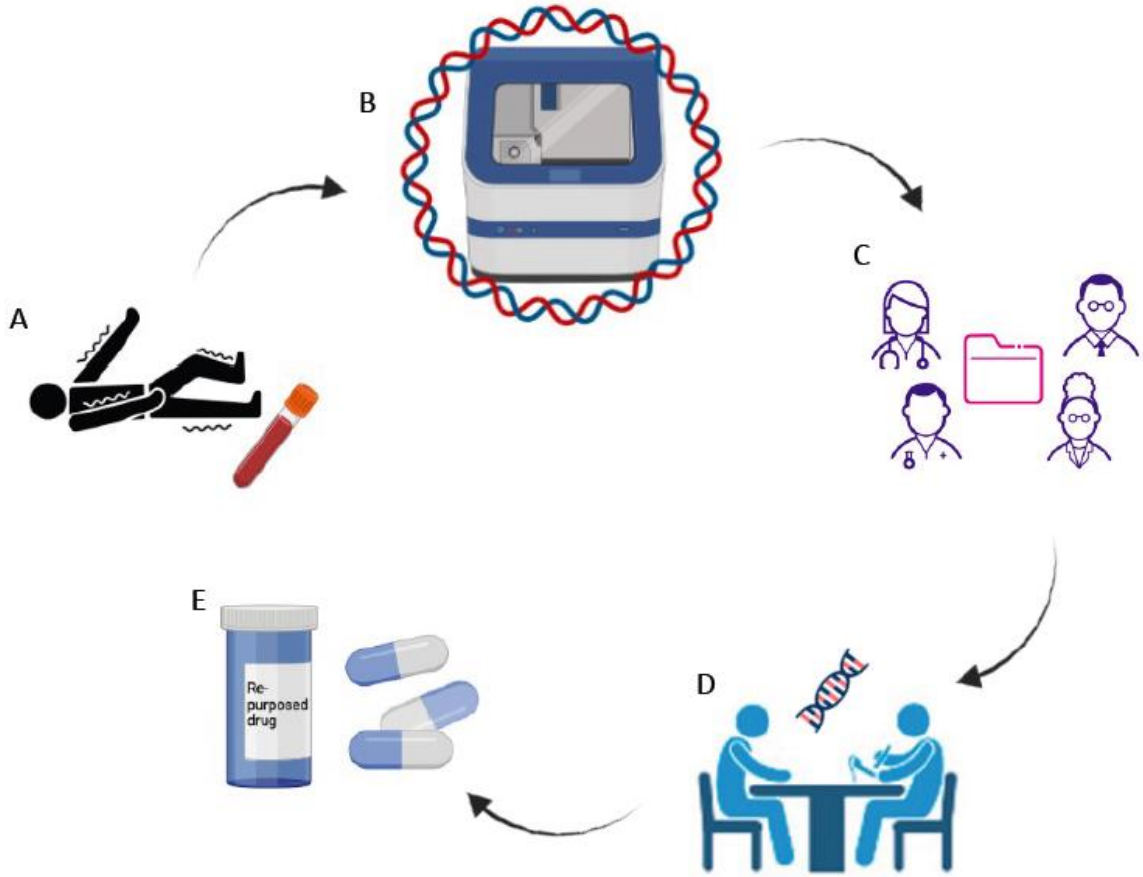

Figure 9.5 Translational research approach for monogenic epilepsies

(A) The patient's research journey begins when they provide informed consent to have a blood sample drawn to better understand the aetiology of their epilepsy. (B) Genomic analysis is performed on the blood sample, as part of the FutureNeuro diagnostic research programme. (C) The clinical significance of candidate genetic variants is discussed at the epilepsy genetics multidisciplinary team meeting, attended by clinicians and translational scientists. (D) If the multidisciplinary team determines that a variant is likely to be pathogenic, the treating clinician meets with the patient and family to discuss the result. (E) If the patient has refractory epilepsy and there is an available precision treatment, the clinician will provide information about the drug and offer the patient the opportunity to trial the drug.

Presentation of my research at several international and national conferences has enabled me to disseminate the findings of my thesis to a wider audience. These meetings not only served as a platform for sharing my research, but also provided opportunities to network and collaborate with clinicians and researchers from other institutions. Such collaborations are crucial in advancing our understanding of the extremely rare genetic epilepsies, as they cannot be studied in isolation.

Epilepsy fellows and neurology registrars who have trained under Professor Delanty recognise his commitment to establishing aetiology in difficult-to-treat epilepsies. Through the course of this research fellowship, I have come to realise that identifying the underlying cause of epilepsy, and implementing rational aetiologic-based treatments may be the best strategy to tackle refractory epilepsy.

Finally, a core aspect of my project involved direct engagement with patients and families, to gather comprehensive medical histories, which are essential for gaining an in-depth understanding of phenotype. These narratives, presented in Chapters 5 and 6, serve as the foundation of my research. Through these interactions, I came to appreciate that monogenic epilepsies are much more than just disorders of difficult-to-treat seizures. Recognising that the "*hidden disability*" can be more debilitating than the seizures themselves, has strengthened my commitment to advocating for the neuropsychiatric and psychosocial needs of people with complex epilepsy.

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11. Appendices

Appendix 1

Ethics (Medical Research) Committee - Beaumont Hospital
Notification of ERC/IRB Approval

This approval is conditional upon receipt of a
Health Research Consent Declaration Committee (HRCDC) declaration

Principal Investigator: **Prof. Norman Delanty, Consultant Neurologist, BH**

REC reference: **21/33**

Protocol Title: **Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies**

Ethics Committee Meeting Date: **26th March 2021**

Final Approval Date: **27th July 2021**

From: **Ethics (Medical Research) Committee - Beaumont Hospital, Beaumont, Dublin 9**

Documents Reviewed

Document and Date	Date Reviewed	Approved
Application Form, V3, 10.7.21	27/7/21	Yes
Signatory Page, PI, 26/1/21	27/7/21	Noted
NB – Email, PI, 3/3/21 – Rationale for conducting this study as a non-interventional trial	27/7/21	Noted
Research Information Leaflet, Adults, V2, 21/5/21	27/7/21	Yes
Research information sheet for 10-18 year olds, V2, 21/5/21	27/7/21	Yes
Research information sheet for parents/ legal guardians V2, 21/5/21	27/7/21	Yes
Patient Consent Form, V2, 21/5/21	27/7/21	Yes
Patient Assent Form, V2, 11/5/21	27/7/21	Yes
Information sheet about everolimus (for health care professionals), no version no.	27/7/21	Noted

Information sheet about everolimus (for patients), no version no.	**27/7/21**	**Noted**
Letter to GPs / Hospital Doctors, no version no. (received 06/21)	**27/7/21**	**Yes**
QOLIE 31, V1.0	**27/7/21**	**Yes**
DPIA	**with DPO**	**with DPO**
GDPR Certs	**27/7/21**	**Noted**
CV / PI	**27/7/21**	**Noted**

Dr. Peter Branagan
ERC/IRB Convenor's Signature
Approval # 1, dated 27th July 2021

Beaumont Hospital
Ethics (Medical Research) Committee

21st September 2021

REC reference: 21/33

Norman Delanty,
Consultant Neurologist,
Beaumont Hospital

To: normandelanty@beaumont.ie; patrickmoloney@beaumont.ie;

Dear Prof. Delanty

REC REF: 21/33 - Directorate - Neurocent
Principal Investigator: Prof. Norman Delanty, Consultant Neurologist, Beaumont Hospital
Study Title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

I acknowledge receipt of the HRCDC declaration for this study and confirm **full** research ethics committee approval is now in place for this research study which seeks to proceed in Beaumont Hospital. (I enclose approval documentation for your files.)

'This submission has been reviewed from an ethical perspective only. It is the responsibility of the PI/sponsor/data controller to ensure and monitor compliance with any relevant legislation in the country where the study is due to take place or any local policy in the site where the study is due to take place.' Receipt of research ethics committee approval is not, and should not be regarded as evidence of compliance with GDPR or the Irish Health Research Regulations 2018 (as amended 2021) The PI/sponsor/data controller is advised to conduct data protection / DPIA reviews of ongoing projects at regular intervals: https://www.beaumontethics.ie/home/dpia_review.htm

It is imperative to report any data protection breaches to dpo@beaumont.ie as soon as you become aware of same; and retain the most up to date copy of the DPIA and make available to the Beaumont Hospital Data Protection Officer on request.

Genetic Data
For your information, the EDPB stated in February 2021 that the possibility of anonymising genetic data remains unresolved and therefore recommended that genetic data should be treated as personal data **–further guidance from European Data Protection Board** is pending.

Post-Approval Process: -
The Beaumont Hospital site sign off process for studies requiring approval is outlined here: - https://www.beaumontethics.ie/home/sign_off.htm
The research study cannot commence until all relevant permissions are in place, and all contracts have been fully executed.

Kind regards

Yours sincerely

Dr. Peter Branagan
Convenor
Ethics (Medical Research) Committee

Appendix 2

(A) Patient Information Leaflet (for adults with capacity)

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

Principal investigator's name: Norman Delanty

Principal investigator's title: Consultant Neurologist
Beaumont Hospital
Professor at Royal College of Surgeons in Ireland (RCSI)
and FutureNeuro: SFI Research Centre

Telephone number of principal investigator: 01 797 4171

Data Controller's Identity: Beaumont Hospital

Data Controller's Contact Details: 01 809 3000

Data Protection Officer's Identity: Mark Granham

Data Protection Officer's Contact Details: 01 809 2162/ dpo@beaumont.ie

You are being invited to take part in a research study to be carried out by the Beaumont Hospital Epilepsy Team.

Before you decide whether or not you wish to take part, you should read the information provided below carefully and, if you wish, discuss it with your family, friends or GP. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.

You don't have to take part in this study. If you decide not to take part it won't affect your future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured it won't affect the quality of treatment you get in the future.

Why is this study being done?

Our genes carry instructions to make molecules called proteins. Proteins perform important functions in our bodies to keep us healthy. Faulty genes are emerging as an important cause of epilepsy, particularly in people with difficult-to-treat seizures.

Tuberous sclerosis complex is a genetic disorder that often causes epilepsy. Seizures in tuberous sclerosis complex are caused by hyperactivity of an important system in the brain, known as the mTOR pathway. Everolimus is a drug that reduces mTOR pathway activity and is used to treat seizures in tuberous sclerosis complex.

GATOR1 complex epilepsy is a genetic disorder caused by faults in the *DEPDC5* and *NPRL2/ 3* genes. Similar to tuberous sclerosis complex, mTOR pathway hyperactivity causes seizures in this disorder. People with GATOR1 complex epilepsy often have difficult-to-treat seizures.

This research study aims to find out if everolimus is an effective and safe treatment for GATOR1 complex epilepsy.

Who is organising and funding this study?

The research is being conducted by Dr. Patrick Moloney under the supervision of Prof. Norman Delanty and Prof. Gianpiero Cavalleri. Dr. Moloney is completing this research in Beaumont Hospital as part of the Royal College of Surgeons in Ireland StAR-MD research programme. His research is sponsored by the Blackrock Clinic, Dublin.

No pharmaceutical companies are funding this research. The researchers are not receiving payment to recruit patients to this study.

Why am I being asked to take part?

You are being asked to participate as your epilepsy is caused by a faulty gene and your seizures have been difficult-to-treat with medications.

How will the study be carried out?

This study takes place at Beaumont Hospital. If you agree to participate, you will be asked to take a drug called everolimus. Everolimus is an approved treatment for seizures in tuberous sclerosis complex. This drug is taken once a day. It will be taken with your usual medications.

We will ask you or a family member to record your seizures in a diary and to complete a questionnaire before starting the drug and at different time points when you are taking the drug.

You will need to attend the outpatient clinic more frequently than before (approximately once every three months). You will see Dr. Patrick Moloney or Prof. Norman Delanty at the clinic. We will take blood samples (around four teaspoons of blood) before starting treatment, 6 weeks after starting treatment and then every 6 months on treatment. The blood samples are needed to measure the level of the drug in your blood. Your blood samples will be stored and destroyed after one week in a laboratory in London.

The study will run over 12 months but if your seizures improve on the new drug, we will continue to prescribe it.

Clinical information related to your epilepsy will be reviewed through your medical record and the Electronic Patient Record here at Beaumont, and stored in a coded, pseudonymised form within an electronic database which can only be accessed by researchers approved by Prof. Delanty. Pseudonymised information cannot be used without access to an individual study code and links your clinical and research information

Video/and or Audio recordings?

There will be no video or audio recordings.

What other treatments are available to me?

You have been asked you to participate in this research study as your seizures have been difficult-to-treat with medications. If you decide that you do not want to participate in the research study or if this new drug does not help your seizures, we will continue to try other medications and therapies for epilepsy.

What are the benefits?

If the new drug helps your seizures, we will continue to prescribe it.

By consenting to be part of this research, you may contribute important new information which may benefit patients in the future. We know that the faulty gene linked to your epilepsy (GATOR1 complex epilepsy) is associated with difficult-to-treat seizures and carries a significant risk of sudden unexpected death from epilepsy (SUDEP). If we find that the treatment helps your seizures, there may be benefits to others with this form of genetic epilepsy.

What are the risks?

Everolimus may cause side-effects. Mouth sores and ulcers are the most common side-effect, seen in around one-third of people taking everolimus. It is important to maintain good oral hygiene when taking everolimus. We recommend using a soft toothbrush and children’s toothpaste. We recommend regular mouth rinses with salt

and water, particularly after eating. If these measures don't help, there are other mouthwashes we can try.

Other rare side-effects include chest infections and rashes. If you experience these, it is important to contact us, as you may need to stop treatment temporarily. Rarely, everolimus causes changes in blood cell counts, including white blood cells which help fight infections. Women of childbearing age should use effective contraception as everolimus may be harmful to unborn babies

The blood sample will be collected using standard clinical practices. We will take around four teaspoons of blood. The blood draw may cause some minor discomfort and potentially some redness, bruising or soreness around the site where the needle is inserted, for perhaps a day or so after. There is also a small risk of infection at the site, however, the likelihood is very low because we follow strict health and safety procedures, including sterilising the area before taking the sample.

What if something goes wrong when I'm taking part in this study?

If you experience any side-effects associated with treatment you can contact Dr. Patrick Moloney by telephone (01 797 4171: answered between 09:00 and 17:00 Monday to Friday) or by email (patrickmoloney@beaumont.ie). The listed side-effects typically resolve after stopping treatment.

Will it cost me anything to take part?

There are no costs associated with your participation in this study.

Is the study confidential?

Will you be writing to my GP?
With your permission, we will correspond with your GP and other healthcare providers involved in your care, so they are aware you are taking everolimus.

Will you be looking at my medical records?
Members of the research team will access details of your treatment and care through your clinical paper record, and electronic records at Beaumont Hospital.

Who else will be looking at my medical records?
Only researchers approved by Prof. Delanty will have access to your medical records.

Will the information about me be kept private and confidential?
Any personal information recorded and stored outside of your hospital medical records will be kept private and confidential. It will not be possible to identify you from this information.

Will information kept about me identify me?
All research work will be pseudonymised. A special code linking your name to collected data will be held by the main investigator Prof. Delanty, to allow updating of clinical information only in respect of the current research project. It will not be used for identification for any other purpose.

Your age, gender, genetic data and information about your epilepsy and seizures will be retained, but no identifying information will be included. However, genetic information is unique to each individual and, therefore, there is some inherent risk of identification. However, we believe this is unlikely to happen. By consenting to the research, you acknowledge that you are aware that such an event could occur.

How long will you keep the information about me?
Data from this specific project will be retained for a maximum of 7 years after the final publication related to the research study.

What will happen to any samples you collect from me?
The blood samples will be sent to an accredited laboratory in London to measure the level of everolimus in your blood. Doctors working in Beaumont Hospital will have access to these results as they will be stored in your medical record.

Will you be publishing the results of this study in medical journals or will you be presenting the results of this study at medical conferences?
The results of our study may be published or presented at a later date. Your name or number will not appear in any publications or presentations. If you wish, we can share the results from the research study with you.

Data Protection

1. We will be using your personal information in our research to help us study if everolimus is an effective treatment for seizures in GATOR1 epilepsy.

2. We wish to process your data under article 6 (1) (f) 'legitimate interests' and article 9 (2) (j) 'for scientific research purposes' of the General Data Protection Regulation 2016 (GDPR). The legitimate interest and scientific research purpose here is to improve treatment options and care for people with epilepsy.

3. Only researchers approved by Prof. Delanty and located at Beaumont Hospital will have access to your medical records. Other named co-investigators will only have access to your data in pseudonymised form, which means your data will be assigned a code for processing.

4. Your coded data will be retained for a maximum of 7 years after the final publication related to the research study.

5. Genetic information is unique to each individual and, therefore, there is some risk of a lack of confidentiality. For example, people not involved in the study who have information about DNA could potentially identify you by comparing your genetic information to information made available in later publications or

presentations. We believe this is unlikely to happen. By consenting to the research, you acknowledge that you are aware that such an event could occur.

6. If you wish to withdraw your consent, you can do so by contacting us by email (patrickmoloney@beaumont.ie) or by phone (01 797 4171). We may arrange a meeting with you to hear your reasons for withdrawal. We will discuss whether some of the data could be kept or used.

   If you decide that you no longer wish to participate whilst we are still in the process of collecting data, you can expect that any data collected from you will be withdrawn and not used in data analysis or in any publication of the outcomes of the research.

   If you decide that you no longer wish to participate once we have begun analysing the data, or when the data analysis has been completed, it becomes much more difficult to remove your data from the overall data set. However, you can expect every effort to be made to remove your data from the project and, as a minimum, any data from which you can be identified will be removed from the project. Prof. Norman Delanty will discuss with you, which data will be removed and the reasons why any remaining data cannot be withdrawn from the project.

   If you would like to exercise your right to be forgotten once the project has completed, all of your personal data from which you can be identified will be deleted from our records in relation to the project.

7. If you wish to lodge a complaint about the research, you can do so through the data protection website: https://www.dataprotection.ie.

8. You have the right to request access to your data and a copy of it. You can make this request by emailing patrickmoloney@beaumont.ie or phoning 01 797 4171.

9. You have the right to restrict or object to processing. You can make this request by contacting us by email (patrickmoloney@beaumont.ie) or by phone (01 797 4171).

10. You have the right to have any inaccurate information corrected or deleted. Please contact us if find any inaccuracies (patrickmoloney@beaumont.ie or 01 797 4171).

11. You have the right to have your personal data deleted. Please contact us if you wish to discuss this (patrickmoloney@beaumont.ie or 01 797 4171).

12. You have the right to have your data moved from one controller to another in a readable format. Please contact us if you wish to discuss this further (patrickmoloney@beaumont.ie or 01 797 4171).

13. There will be no automated decision making.

14. We will contact you if we have any plans to further process your data and we will provide information on other purposes if these arise.

Consent to future uses

Some of the data collected in this study may be useful for future research. This data may be used for future research to learn more about the treatment of epilepsy. This data will be stored in a pseudonymised form, which means your data will be assigned a code for processing.

If any possible future research arises for which you may be eligible, you would be contacted by researchers and your consent to participate would be sought. Any future studies involving your data will be subject to the approvals applied for from a Research Ethics Committee.

You can change your mind about taking part in this study or any future research at any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured It won't affect the quality of treatment you get in the future.

Where can I get further information?

If you have any further questions about the study now or at any time in the future, please contact:

Patrick Moloney or Norman Delanty
Department of Neurology,
Beaumont Hospital,
Dublin 9

01 797 4171 (phone is answered between 09:00 and 17:00 Monday to Friday) or
patrickmoloney@beaumont.ie

(B) Patient Information Leaflet (for 14 to 17-year-olds)

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

Principal investigator's name: Norman Delanty

Principal investigator's title: Consultant Neurologist
Beaumont Hospital
Professor at Royal College of Surgeons in Ireland (RCSI)
and FutureNeuro: SFI Research Centre

Telephone number of principal investigator: 01 797 4171

Data Controller's Identity: Beaumont Hospital

Data Controller's Contact Details: 01 809 3000

Data Protection Officer's Identity: Mark Granham

Data Protection Officer's Contact Details: 01 809 2162/ dpo@beaumont.ie

You are being invited to take part in a research study to be carried by the Beaumont Hospital Epilepsy Team.

Before you decide whether or not you wish to take part, you should read this information sheet and discuss it with your family, friends or GP. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

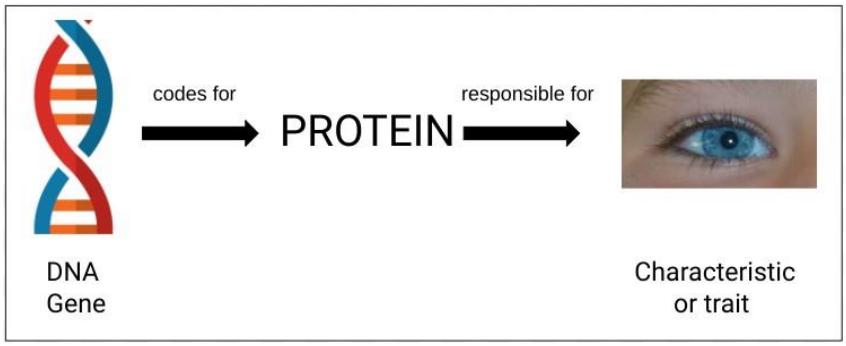
You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you.

You don't have to take part in this study. If you decide not to take part it won't affect your future care at the epilepsy clinic.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still drop out. You don't have to give us a reason. If you do drop out, rest assured it won't affect the quality of treatment you get in the future.

Why is this study being done?

Our genes influence our physical appearance and health. Each gene has a special job. Each gene carries specific instructions for making proteins. Proteins are the building blocks for making bones, muscles, blood and nerves. Some illnesses are caused by genes that don't work as they should.



Epilepsy may be caused by a faulty gene. Tuberous sclerosis complex is a common cause of genetic epilepsy. Seizures in tuberous sclerosis complex are caused by overactivity of an important system in the brain, known as the mTOR pathway. Everolimus is a drug that reduces mTOR pathway activity and is used to treat seizures in tuberous sclerosis complex.

GATOR1 complex epilepsy is very similar to tuberous sclerosis complex. Changes in three genes (*DEPDC5, NPRL2* and *NPRL3)* cause this type of epilepsy. The mTOR pathway is also overactive in this type of epilepsy. People with GATOR1 complex epilepsy often have seizures that are difficult-to-treat with medicines.

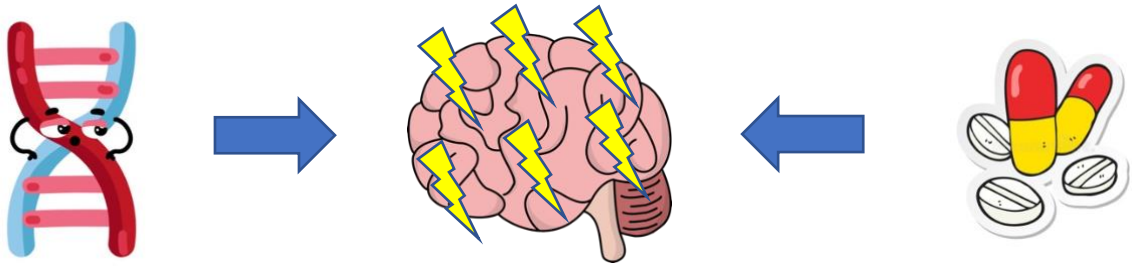
This research study aims to find out if everolimus is an effective and safe medicine for GATOR1 complex epilepsy.

Who is organising and funding this study?

Dr. Patrick Moloney is completing this research under the supervision of Prof. Norman Delanty and Prof. Gianpiero Cavalleri. Dr. Moloney completing this research as part of the Royal College of Surgeons in Ireland StAR MD programme. His MD is sponsored by the Blackrock Clinic, Dublin.

Why am I being asked to take part?

You are being asked to take part as your epilepsy is caused by a fault in a gene and your seizures have been difficult-to-treat with medicines.



What will happen to me if I agree to take part?

If you agree to take part you will be asked to take a drug called everolimus. Everolimus is used to treat seizures in a type of epilepsy called tuberous sclerosis complex. This drug is taken once a day. It will be taken with your usual medicines.

We will ask you to record your seizures in a diary and to answer some questions before starting the medicine and at different times after starting the medicine.

We will ask you to attend the clinic more often than before (around once every three months). You will see Dr. Patrick Moloney or Prof. Norman Delanty at the clinic. We will take blood samples (around four teaspoons of blood) before starting the new medicine and during the clinic visits to measure the level of the medicine in your blood. The study will run over one year but if your seizures improve, we will continue to give you the new medicine.

We will learn about your epilepsy through your medical chart and the computer system here at Beaumont Hospital. This information about you will be stored on a very secure computer. Researchers on the epilepsy team will need a special code to access this information.

What other treatments are available to me?

You have been asked you to take part in this research study as your seizures have been difficult- to-treat with medicines. If you decide that you do not want to take part or if this new medicine does not help your seizures, we will try other medicines in the future.

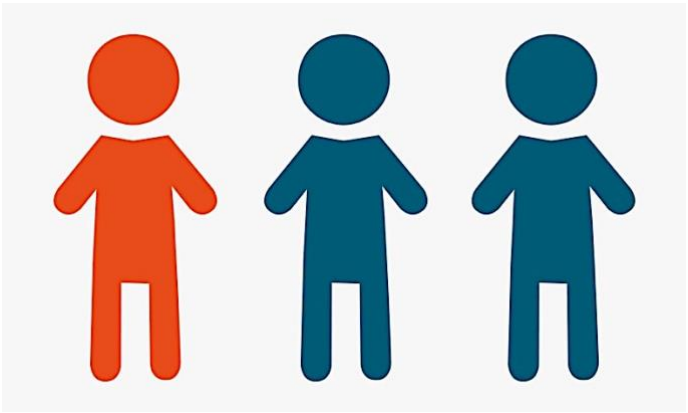
What are the benefits?

If the new medicine helps your seizures, we will continue to prescribe it.

By agreeing to take part in this research, you may help other people with epilepsy who have seizures that are difficult-to-treat with medicines.

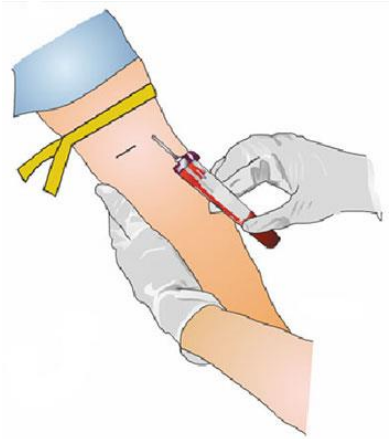
What are the risks?

Everolimus can cause mouth sores. Around one in three of people taking everolimus get mouth sores. It is important to keep your teeth and mouth very clean. We recommend using a soft toothbrush and mild toothpaste. We recommend regular mouth rinses with salt and water, especially after eating. There are other mouthwashes we can try if these steps don't help.



Other rare side-effects include chest infections and rashes. If you get these, it is important to call us, as you may need to stop the medicine. We need to take blood samples to be sure your body is responding well to the medicine.

The blood test may cause mild pain and some redness or soreness around the site where the needle is inserted, for perhaps a day or so after. There is a very small risk of infection where we insert the needle. However, this is very unlikely as make sure the area is very clean before taking the blood sample.



What if something goes wrong when I'm taking part in this study?

If you experience any problems on the new medicine, you can contact Dr. Patrick Moloney by telephone (01 797 4171: answered between 09:00 and 17:00 Monday to Friday) or by email (patrickmoloney@beaumont.ie).

Will it cost me anything to take part?

There are no costs associated with taking part in this study.

Is the study confidential?

Will you be writing to my GP?
With your permission, we will write a letter to your GP so he/she is aware you are taking a new medicine.

Will you be looking at my medical records?
Members of the research team will get information about your epilepsy through your medical chart, and the computer system at Beaumont Hospital.

Who else will be looking at my medical records?
Dr. Patrick Moloney and Prof. Norman Delanty will have access to your medical records.

Will the information about me be kept private and confidential?
Any personal information recorded and stored outside of your hospital medical records will be kept private.

Will information kept about me identify me?
Your age, gender, and information about your genes and seizures will be stored on a very secure computer. A special code linking your name to the information we collect will be held by Prof. Delanty.

How long will you keep the information about me?
We will keep information about you for a maximum of 7 years after the final publication related to the research.

What will happen to any samples you collect from me?
The blood samples will be sent to a laboratory in London. Doctors working in Beaumont Hospital will have access to these results as they will be stored in your medical chart.

Will you be publishing the results of this study in medical journals or will you be presenting the results of this study at medical conferences?
The results of our study may be published or presented at a later date. Your name will not appear in any publications or presentations. If you wish, we can share the results from the study with you.

What will happen if you tell us something or we see something that worries us about your situation at home?

If we have any concerns, we will discuss this with you. We may need to report our concerns to appropriate services.

Data Protection

1. We will be using your personal information to study how good a medicine is at treating seizures caused by an abnormal gene.

2. We wish to use your personal information to improve treatment options and care for people with epilepsy.

3. Only researchers approved by Prof. Delanty will have access to your medical records.

4. We will keep your personal information for a maximum of 7 years after the final publication related to the research.

5. Your genes are unique. There is a tiny risk that someone could identify you from your genetic information.

6. It is possible to withdraw from the study by contacting us by email (*patrickmoloney@beaumont.ie*) or by phoning us (01 797 4171). We may arrange a meeting with you to hear your reasons for withdrawal. We will discuss whether some of the data could be kept or used.

   If you decide that you no longer wish to take part when we are still collecting information about you, you can expect that any information about you will be withdrawn and not used in data analysis or in any publication about the results of the research.

   If you decide that you no longer wish to take part once we have begun analysing the information we have collected, or when the data analysis has been completed, it becomes much more difficult to remove your data. However, you can expect every effort to be made to remove your data from the project and, as a minimum, any data from which you can be identified will be removed from the project. Prof. Norman Delanty will discuss with you, which data will be removed and the reasons why any remaining data cannot be withdrawn from the project.

   If you would like to exercise your right to be forgotten once the project has completed, all of your personal data from which you can be identified will be deleted from our records in relation to the project.

7. If you wish to make a complaint about the research, you can do so through the data protection website: https://www.dataprotection.ie.

8. You have the right to get a copy of your personal information collected for the study. You can make this request by emailing us (*patrickmoloney@beaumont.ie*) or phoning us (01 797 4171).

9. You have the right to block us using your personal information. You can make this request by contacting us by email (*patrickmoloney@beaumont.ie*.) or by phoning us (01 797 4171).

10. You have the right to have any wrong information about you corrected or removed. Please contact us if you wish to make this request (*patrickmoloney@beaumont.ie* or 01 797 4171).

11. You have the right to have your personal information deleted. Please contact us if you wish to discuss this (*patrickmoloney@beaumont.ie* or 01 797 4171).

12. We will contact you if we have any plans to use your personal information for other research.

Consent to future uses

Some of the information collected in this study may be useful for future research. This information may be used to learn more about the treatment of epilepsy.

If future research opportunities arise, we will contact you and look for your permission to include you in this.

You can change your mind about taking part in this study or any future research at any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured It won't affect the quality of treatment you get in the future.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.
If you need any further information now or at any time in the future, please contact:

Patrick Moloney
Department of Neurology,
Beaumont Hospital,
Dublin 9

01 797 4171 (phone is answered between 09:00 and 17:00 Monday to Friday) or patrickmoloney@beaumont.ie

(C) Information Leaflet (for Parents/ Legal Guardians)

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

Principal investigator's name: Norman Delanty

Principal investigator's title: Consultant Neurologist
Beaumont Hospital
Professor at Royal College of Surgeons in Ireland (RCSI)
and FutureNeuro: SFI Research Centre

Telephone number of principal investigator: 01 797 4171

Data Controller's Identity: Beaumont Hospital

Data Controller's Contact Details: 01 809 3000

Data Protection Officer's Identity: Mark Granham

Data Protection Officer's Contact Details: 01 809 2162/ dpo@beaumont.ie

Your child has been invited to take part in a research study to be carried out by the Beaumont Hospital Epilepsy Team.

Before you decide whether or not you want your son or daughter to participate, you should read the information provided below carefully and, if you wish, discuss it with your family, friends or GP. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study. This process is known as 'Informed Consent'.

Your child does not have to take part in this study. If you decide that you don't want your child to participate it won't affect his or her future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you decide to opt out, rest assured it won't affect the quality of treatment he or she gets in the future.

Why is this study being done?

Our genes carry instructions to make molecules called proteins. These proteins perform important functions in our bodies to keep us healthy. Genetic abnormalities are emerging as an important cause of epilepsy, particularly in people with difficult-to-treat seizures.

Tuberous sclerosis complex is a common cause of genetic epilepsy. Seizures in tuberous sclerosis complex are caused by overactivity of an important system in the brain, known as the mTOR pathway. Everolimus is a drug that reduces mTOR pathway activity and is used to treat seizures in tuberous sclerosis complex.

GATOR1 complex epilepsy is very similar to tuberous sclerosis complex. GATOR1 complex epilepsy is caused by faults in three gene (*DEPDC5* or *NPRL2/ 3)*. Similar to tuberous sclerosis complex, mTOR pathway hyperactivity causes seizures in this disorder. People with GATOR1 complex epilepsy often have difficult-to-treat seizures.

This research study aims to find out if everolimus is an effective and safe treatment for GATOR1 complex epilepsy.

Who is organising and funding this study?

The research is being conducted by Dr. Patrick Moloney under the supervision of Prof. Norman Delanty and Prof. Gianpiero Cavalleri. Dr. Moloney is completing this research in Beaumont Hospital as part of the Royal College of Surgeons in Ireland StAR-MD research programme. His research is sponsored by the Blackrock Clinic, Dublin.

No pharmaceutical companies are funding this research. The researchers are not receiving payment to recruit patients to this study.

Why is your child being asked to take part?

Your child is being asked to participate as his/her epilepsy is caused by a faulty gene and his/her seizures have been difficult-to-treat with medications.

How will the study be carried out?

This study takes place at Beaumont Hospital. If your child participates in this study, we will ask him/her to take a new medication (everolimus). Everolimus is an approved treatment for seizures in tuberous sclerosis complex. This drug is taken once daily and will be taken with your child's usual medications. The study will run over 12 months but if your child's seizures improve on everolimus we will continue to prescribe it.

You will need to record your child's seizures in a diary and to help him/her complete a questionnaire before starting the drug and at different time points after starting the drug.

We will ask you and your child to attend the outpatient clinic more frequently than before (approximately once every three months). You will see Dr. Patrick Moloney or Prof. Norman Delanty at the clinic and they will have access to your child's medical records. We will take blood samples (around four teaspoons of blood) before starting treatment and during clinic visits 6 weeks after starting treatment and then every 6 months on treatment to measure the level of the drug. Blood samples taken to measure the level of everolimus will be stored and destroyed after one week in a laboratory in London.

Clinical information related to your child's epilepsy will be reviewed through his/her medical record and the Electronic Patient Record here at Beaumont and stored in a coded, pseudonymised form within an electronic database which can only be accessed by researchers approved by Prof. Delanty.

Pseudonymised information is information which cannot be used without access to an individual study code that can only be used by specific members of the epilepsy team to link clinical and research information. These members of the epilepsy team are persons who are approved in advance by Prof. Delanty to carry out these tasks, confidentially.

Video/and or Audio recordings?

There will be no video or audio recordings.

What other treatments are available?

We have asked your child to participate in this research study as his/her seizures have been difficult to treat with medications. If you and your child decide that you don't want to take part or if everolimus does not help with your child's seizures, we will continue to try other medications and therapies for epilepsy.

What are the benefits?

If the new drug helps your child's seizures, we will continue to prescribe it.

By consenting to be part of this research, you may contribute important new information which may benefit patients in the future. We know that your child's genetic abnormality causes difficult-to-treat seizures and carries a significant risk of sudden unexpected death from epilepsy (SUDEP). If we find that the treatment helps with seizures, there may be benefits to others with this form of genetic epilepsy.

What are the risks?

Everolimus may cause side-effects. Mouth sores and ulcers are the most common side-effect, seen in around one-third of people taking everolimus. It is important to maintain good oral hygiene when taking everolimus. We recommend using a soft toothbrush and children's toothpaste. We recommend regular mouth rinses with salt and water, particularly after eating. If these measures don't help, there are other mouthwashes we can try.

Other rare side-effects include chest infections and rashes. If your child experiences these, it is important to contact us, as we may need to stop treatment temporarily. Rarely, everolimus causes reduced blood cell counts, including white blood cells which help fight infections. Women of childbearing age should use effective contraception as everolimus may be harmful to unborn babies

The blood sample will be collected using standard clinical practices. We will draw around four teaspoons of blood. The blood draw may cause some minor discomfort and potentially some redness or soreness around the site where the needle is inserted, for perhaps a day or so after. There is also a small risk of infection at the site, however, the likelihood is very low because we follow strict health and safety procedures (including sterilising the area before taking a sample).

What if something goes wrong when I'm taking part in this study?

If your child experiences any side-effects associated with treatment you can contact Dr. Patrick Moloney by telephone (01 797 4171: answered between 09:00 and 17:00 Monday to Friday) or by email (patrickmoloney@beaumont.ie). The listed side-effects typically resolve after stopping treatment.

Will it cost me anything to take part?

There are no costs associated with your participation in this study.

Is the study confidential?

Will you be writing to my child's GP?
With your permission, we will correspond with your child's GP and other healthcare providers involved in his/her care, so they are aware he/she is taking everolimus.

Will you be looking at my child's medical records?
Members of the research team will access details of your child's treatment and care through his/her clinical paper record, and electronic records at Beaumont Hospital.

Who else will be looking at his/her medical records?
Only researchers approved by Prof. Delanty will have access to his/her medical records.

Will the information about my child be kept private and confidential?
Any personal information recorded and stored outside of his/her hospital medical records will be kept private and confidential. It will not be possible to identify him/her from this information.

Will information kept about my child identify him/her?
All research work will be pseudonymised. A special code linking his/her name to the collected data will be held by the main investigator Prof. Delanty, to allow updating of clinical information only in respect of the current research project. It will not be used for identification for any other purpose.

Your child's age, gender, genetic data and information about his/her epilepsy and seizures will be retained, but no identifying information will be included. However, genetic information is unique to each individual and, therefore, there is some inherent risk of identification. However, we believe this is unlikely to happen. By consenting to the research, you acknowledge that you are aware that such an event could occur.

How long will you keep the information about me?
Data from this specific project will be retained for a maximum of 7 years after the final publication related to the research study.

What will happen to any samples you collect from my child?
The blood samples will be sent to an accredited laboratory in London to measure the level of everolimus in blood. Doctors working in Beaumont Hospital will have access to these results as they will be stored in your child's medical record.

Will you be publishing the results of this study in medical journals or will you be presenting the results of this study at medical conferences?
The results of our study may be published or presented at a later date. Your child's name will not appear in any publications or presentations. If you wish, we can share the results from the research study with you.

What will happen if your child reveals to the investigators, he/she is at risk of harm?

If your child reveals to the investigators that he/she is at risk of harm or if there is evidence of significant risk of harm, we are required to report this to appropriate services. This will happen following discussion with your child.

Data Protection

1. We will be using your child's personal information in our research to help us study if everolimus is an effective treatment for seizures in GATOR1 complex epilepsies.

2. We wish to process your child's data under article 6 (1) (f) 'legitimate interests' and article 9 (2) (j) 'for scientific research purposes' of the General Data Protection Regulation 2016 (GDPR). The legitimate interest and scientific

research purpose here is to improve treatment options and care for people with epilepsy.

3. Only researchers approved by Prof. Delanty and located at Beaumont Hospital will have access to your child's medical records. Other named co-investigators will only have access to his/her data in pseudonymised form, which means his/her data will be assigned a code for processing.

4. Your child's coded data will be retained for a maximum of 7 years after the final publication related to the research study.

5. Genetic information is unique to each individual and, therefore, there is some risk of a lack of confidentiality. For example, people not involved in the study who have information about DNA could potentially identify your child by comparing his/her genetic information to information made available in later publications or presentations. We believe this is unlikely to happen. By consenting to the research, you acknowledge that you are aware that such an event could occur.

6. If you wish to withdraw your consent, you can do so by contacting us by email (patrickmoloney@beaumont.ie) or by phone (01 797 4171). We may arrange a meeting with you to hear your reasons for withdrawal. We will discuss whether some of the data could be kept or used.

   If you decide to withdraw your consent whilst we are still in the process of collecting data, you can expect that any data collected about _____ (*name of participant*) will be withdrawn and not used in data analysis or in any publication of the outcomes of the research.

   If you decide to withdraw your consent once we have begun analysing the data, or when the data analysis has been completed, it becomes much more difficult to remove _____'s (*name of participant*) data from the overall data set. However, you can expect every effort to be made to remove his/her data from the project and, as a minimum, any data from which he/she can be identified will be removed from the project. Prof. Norman Delanty will discuss with you, which data will be removed and the reasons why any remaining data cannot be withdrawn from the project.

   If you would like to exercise _____'s (*name of participant*) right to be forgotten once the project has completed, all of his/her personal data from which he/she can be identified will be deleted from our records in relation to the project.

7. If you wish to lodge a complaint about the research, you can do so through the data protection website: https://www.dataprotection.ie.

8. You have the right to request access to your child's data and a copy of it. You can make this request by emailing patrickmoloney@beaumont.ie or by phoning 01 797 4171.

9. You have the right to restrict or object to processing. You can make this request by contacting us by email (patrickmoloney@beaumont.ie) or by telephone (01 797 4171).

10. You have the right to have any inaccurate information corrected or deleted. Please contact us if find any inaccuracies (patrickmoloney@beaumont.ie or 01 797 4171).

11. You have the right to have your child's personal data deleted. Please contact us if you wish to discuss this (patrickmoloney@beaumont.ie or 01 797 4171).

12. You have the right to have your child's data moved from one controller to another in a readable format. Please contact us if you wish to discuss this further (patrickmoloney@beaumont.ie or 01 797 4171).

13. There will be no automated decision making.

14. We will contact you if we have any plans to further process your child's data and we will provide information on other purposes if these arise.

Consent to future uses

Some of the data collected in this study may be useful for future research. This data may be used for future research to learn more about the treatment of epilepsy. This data will be stored in a pseudonymised form, which means your child's data will be assigned a code for processing.

If any possible future research arises for which your child may be eligible, you would be contacted by researchers and consent to participate would be sought. Any future studies involving your child's data will be subject to the approvals applied for from a Research Ethics Committee.

You can change your mind about participation in this study or any future research at any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured It won't affect the quality of treatment your child gets in the future.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.
If you need any further information now or at any time in the future, please contact:

Patrick Moloney
Department of Neurology,
Beaumont Hospital,
Dublin 9
01 797 4171 or patrickmoloney@beaumont.ie

(D) Patient Information Leaflet (for adult's lacking capacity)

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

Principal investigator's name: Norman Delanty

Principal investigator's title: Consultant Neurologist
Beaumont Hospital
Professor at Royal College of Surgeons in Ireland (RCSI)
and FutureNeuro: SFI Research Centre

Telephone number of principal investigator: 01 797 4171

Data Controller's Identity: Beaumont Hospital

Data Controller's Contact Details: 01 809 3000

Data Protection Officer's Identity: Mark Granham

Data Protection Officer's Contact Details: 01 809 2162/ dpo@beaumont.ie

You are being invited to take part in a research study by the Beaumont Hospital Epilepsy Team.

Before you decide if you want to take part, you should read this information and discuss it with your family, friends or GP. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

Important things to know….

- You get to decide if you want to take part.
- You can say 'No' or you can say 'Yes'.
- No one will be upset if you say 'No'.
- If you say 'Yes' you can always say 'No' later.
- You can say 'No' at any time.
- We would still take good care of you no matter what you decide.

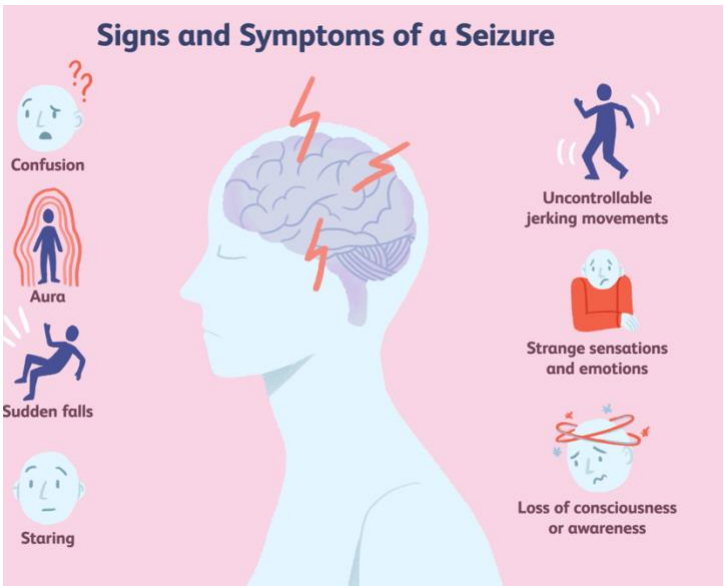
What is a research study?

A research study is what you do when you want to learn about something or find out something new.

This form talks about our research and your options about taking part in the research. We want to answer any questions that you have. You can ask questions at any time.

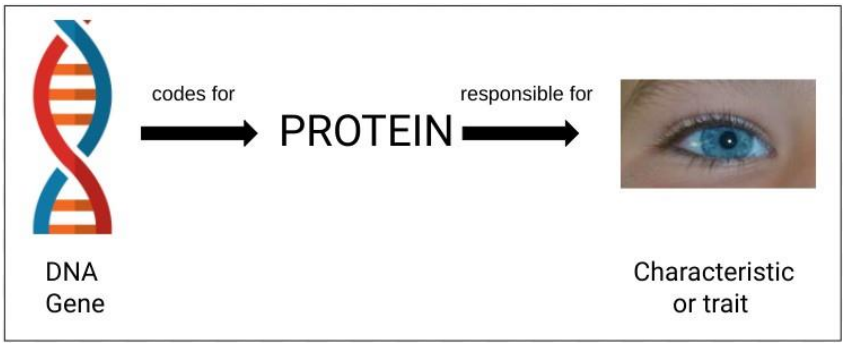
Why is this study being done?

A seizure happens when your brain takes a little break. During a seizure a person can cry out, go stiff, fall down, and shake their arms and legs. People often don't remember having a seizure. People with epilepsy can have seizures at any time.



We use medicines to stop seizures from happening. For some people it is difficult to treat seizures with medicines.

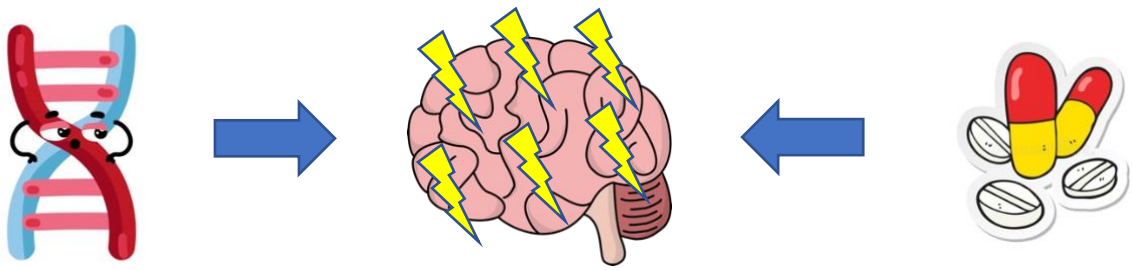
Our genes influence how we look and how we feel. Each gene has a special job. Each gene carries a special message for making proteins. Proteins are the building blocks for making bones, muscles, blood and nerves. Some illnesses are caused by genes that don't work as they should.



Sometimes, epilepsy is caused by a faulty gene. We know that your epilepsy is caused by a faulty gene. This research aims to find out if a medicine that targets the faulty gene can help your seizures.

Why am I being asked to take part?

You are being asked to take part as your epilepsy is caused by a faulty gene and your seizures have been difficult-to-treat with medicines.



How will the study be carried out?

We will ask you to take a new medicine called everolimus. Everolimus is used to treat seizures in a type of epilepsy called tuberous sclerosis complex. This drug is taken once a day. It will be taken with your usual medicines.

We will ask you and to records your seizures in a diary and to answer some questions before starting the medicine and at different times after starting the medicine.

We will take blood samples before starting, 6 weeks after starting and then every 6 months when you are taking the new medicine. The blood samples are needed to measure the level of the medicine in your blood.

We will learn about your epilepsy through your medical chart and the computer system here at Beaumont Hospital. This information about you will be stored on a

very secure computer. Researchers on the epilepsy team will need a special code to access this information.

We will ask you to attend the clinic more often than before (around once every three months). You will see Dr. Patrick Moloney or Prof. Norman Delanty at the clinic. The study will run over one year but if your seizures improve, we will continue to give you the new medicine.

What other treatments are available to me?

You have been asked you to take part in this research study as your seizures have been difficult-to-treat with medicines. If you decide that you do not want to take part or if this new medicine does not help your seizures, we will try other medicines in the future.

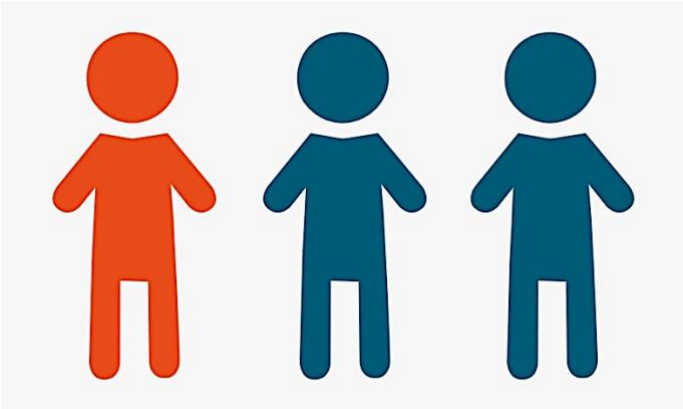
What are the benefits?

If the new medicine helps your seizures, we will continue to prescribe it.

By agreeing to take part in this research, you may help other people with epilepsy who have seizures that are difficult-to-treat with medicines.

What are the risks?

Everolimus can cause mouth sores. Around one in three people taking the medicine get mouth sores. It is important to keep your teeth and mouth very clean. We recommend using a soft toothbrush and mild toothpaste. We recommend regular mouth rinses with salt and water, especially after eating. There are other mouthwashes we can try if this doesn't help.



Sometimes the medicine can cause a chest infection or rash. If you get these, it is important to call us, as you may need to stop the medicine. Blood samples need to be taken so we can be sure that your body is responding well to the new medicine.

The blood test may cause mild pain and some redness or soreness around the site where the needle is inserted, for perhaps a day or so after. There is a very small risk of infection where we insert the needle. However, this is very unlikely as make sure the area is very clean before taking the blood sample.

What if something goes wrong when I'm taking part in this study?

If you experience any problems on the new medicine, you can contact Dr. Patrick Moloney by telephone (017974171: answered between 09:00 and 17:00 Monday to Friday) or by email (patrickmoloney@beaumont.ie).

Will it cost me anything to take part?

There are no costs associated with taking part in this study.

Is the study confidential?

Will you be writing to my GP?
With your permission, we will write a letter to your GP, so he/she is aware you are taking a new medicine.

Will you be looking at my medical records?
Members of the team will get information about your epilepsy through your medical chart, and the computer system at Beaumont Hospital.

Who else will be looking at my medical records?
Dr. Patrick Moloney and Prof. Norman Delanty will have access to your medical records.

Will the information about me be kept private and confidential?
Any personal information recorded and stored outside of your hospital medical records will be kept private.

Will information kept about me identify me?
Your age, gender, and information about your genes and seizures will be stored on a very secure computer. A special code linking your name to the information we collect will be held by Prof. Delanty.

How long will you keep the information about me?
We will keep information about you for a maximum of 7 years after the final publication related to the research.

What will happen to any samples you collect from me?
The blood samples will be sent to a laboratory in London. Doctors working in Beaumont Hospital will have access to these results as they will be stored in your medical chart.

Will you be publishing the results of this study in medical journals or will you be presenting the results of this study at medical conferences?

The results of our study may be published or presented at a later date. Your name will not appear in any publications or presentations. If you wish, we can share the results from the study with you.

What will happen if you tell us something or we see something that worries us about your situation at home?

If we have any concerns, we will discuss this with you. We may need to report our concerns to appropriate services.

Data Protection

- We will be using your personal information to study how good a medicine is at treating seizures caused by a faulty gene.
- We wish to use your personal information to improve treatment options and care for people with epilepsy.
- Only researchers approved by Prof. Delanty will have access to your medical records.
- We will keep your personal information for a maximum of 7 years after the final publication related to the research.
- Your genes are unique. There is a tiny risk that someone could identify you from your genetic information.
- It is possible to withdraw from the study by contacting us by email (*patrickmoloney@beaumont.ie*.) or by phoning us (01 797 4171). We may arrange a meeting with you to hear your reasons for withdrawing. We will discuss whether some of the information about you could be kept or used.
- If you decide to withdraw when we are still collecting information about you, you can expect that all information about you will be withdrawn and not used in our research or in any publication about the results of the research.
- If you decide to withdraw once we have begun analysing your information, or when the analysis has been completed, it becomes much more difficult to remove your information from our records. However, you can expect every effort to be made to remove your information from the project. Prof. Norman Delanty will discuss with you, what information will be removed and the reasons why some information cannot be removed from the project.
- If you would like to exercise your right to be forgotten once the research has been completed, all of your data from which you can be identified will be deleted from our records.

- If you wish to make a complaint about the research, you can do so through the data protection website: https://www.dataprotection.ie.

- You have the right to get a copy of your personal information collected for the study. You can make this request by emailing us (*patrickmoloney@beaumont.ie*.) or phoning us (01 797 4171).

- You have the right to block us using your personal information. You can make this request by contacting us by email (*patrickmoloney@beaumont.ie*.) or by telephone (01 797 4171).

- You have the right to have any wrong information about you corrected or removed. Please contact us if you wish to make this request (*patrickmoloney@beaumont.ie* or 01 797 4171).

- You have the right to have your personal information deleted. Please contact us if you wish to discuss this (*patrickmoloney@beaumont.ie* or 01 797 4171).

- We will contact you if we have any plans to use your personal information for other research.

Future research

Some of the information collected in this study may be useful for future research. This information may be used to learn more about the treatment of epilepsy.

If future research opportunities arise, we will contact you and ask if you want to take part.

You can change your mind about taking part in this study or any future research at any time you like. Even if the study has started, you can still drop out. You don't have to give us a reason. If you do drop out, rest assured It won't affect the quality of treatment you get in the future.

Where can I get further information?

Patrick Moloney
Department of Neurology,
Beaumont Hospital,
Dublin 9
01 797 4171 (phone is answered between 09:00 and 17:00 Monday to Friday)
patrickmoloney@beaumont.ie

(E) PATIENT CONSENT FORM

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

I have read and understood the **Information Leaflet** about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	**Yes** □	**No** □
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	**Yes** □	**No** □
I am aware of the potential risks, benefits and alternatives of this research study.	**Yes** □	**No** □
I give permission for researchers to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	**Yes** □	**No** □
I have been given a copy of the Information Leaflet and this completed consent form for my records.	**Yes** □	**No** □
I consent to take part in this research study having been fully informed of the risks, benefits and alternatives.	**Yes** □	**No** □
I give informed explicit consent to have my data processed as part of this research study.	**Yes** □	**No** □
I consent to be contacted by researchers as part of this research study.	**Yes** □	**No** □
I consent for my GP and other doctors involved in my care to be informed about my participation in this research	**Yes** □	**No** □
I consent to have blood samples taken to check the level of everolimus in blood and to monitor for complications		

FUTURE CONTACT [please choose one or more as you see fit]		
I consent to be re-contacted by researchers about possible future research **related** to the current study for which I may be eligible.	**Yes** □	**No** □
I consent to be re-contacted by researchers about possible future research **unrelated** to the current study for which I may be eligible.	**Yes** □	**No** □

STORAGE AND FUTURE USE OF INFORMATION		
RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]		
I give permission for data to be stored for *possible future research* ***related*** to the current study ***only if participant consent is obtained*** at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes □	No □
I give permission for data to be stored for *possible future research* ***unrelated*** to the current study ***only if participant consent is obtained*** at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes □	No □

Patient Name (Block Capitals)	Patient Signature	Date

Translator Name (Block Capitals)	Signature and Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals)	Qualifications	Signature	Date

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

(F) CONSENT FORM FOR PARENT/LEGAL GUARDIAN

Study title: Everolimus for drug-resistant seizures associated with GATOR1 complex-related epilepsies

I have read and understood the **Information Leaflet** about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	**Yes** □	**No** □
I understand that _____ (*name of study participant*) does not have to take part in this study and that opting out at any time is okay. I understand that I do not have to give a reason for opting out and I understand that opting out won't affect his/her future medical care.	**Yes** □	**No** □
I am aware of the potential risks, benefits and alternatives of this research study.	**Yes** □	**No** □
I give permission for researchers to look at his/her medical records to get information. I have been assured that information about him/ her will be kept private and confidential.	**Yes** □	**No** □
I have been given a copy of the Information Leaflet and this completed assent form for my records.	**Yes** □	**No** □
I give my consent for _____ (*name of study participant*) to take part in this research study having been fully informed of the risks, benefits and alternatives.	**Yes** □	**No** □
I give my consent to have his/her data processed as part of this research study.	**Yes** □	**No** □
I agree to be contacted by researchers as part of this research study.	**Yes** □	**No** □
I agree for his/her GP and other doctors involved in his/her care to be informed about his/her participation in this research	**Yes** □	**No** □
I agree for blood samples to be taken to check the level of everolimus and to monitor for complications		

FUTURE CONTACT [please choose one or more as you see fit]		
I agree to be re-contacted by researchers about possible future research **related** to the current study for which _____ may be eligible.	**Yes** □	**No** □
I agree to be re-contacted by researchers about possible future research **unrelated** to the current study for which _____ may be eligible.	**Yes** □	**No** □

STORAGE AND FUTURE USE OF INFORMATION		
RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]		
I give permission for data to be stored for *possible future research* ***related*** to the current study ***only if consent/assent is obtained*** at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes □	No □
I give permission for data to be stored for *possible future research* ***unrelated*** to the current study ***only if consent/assent is obtained*** at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes □	No □

Patient Name (Block Capitals)	Patient Signature	Date

Translator Name (Block Capitals)	Signature and Date

Legal Representative/Guardian Name	Signature and Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals)	Qualifications	Signature	Date

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

Appendix 3

HRCDC
Health Research Consent Declaration Committee

Teach Grattan
67-72 Sráid an Mhóta Íochtarach
Baile Átha Cliath 2
D02 H638
Éire

Grattan House
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F: 353 1 661 2335
E: info@hrcdc.ie

www.hrcdc.ie

PRIVATE AND CONFIDENTIAL

Prof. Norman Delanty
Department of Neurology,
Beaumont Hospital, Beaumont Road,
Dublin 9, Ireland

By email only: normandelanty@beaumont.ie
Cc by email only: *Dr. Patrick Moloney, patrickmoloney@beaumont.ie*

17th September 2021

Dear Prof. Delanty,

RE:	**Application:**	"Everolimus for drug-resistant seizures associated with GATOR1 complex epilepsies"
	Reference ID:	21-012-AF1
	Data Controller(s):	Beaumont Hospital, Dublin
	Decision:	Conditional Declaration

Thank you for your application to the HRCDC seeking a consent declaration on behalf of Beaumont Hospital, Dublin. The HRCDC convened on 7th September 2021 and reviewed the above referenced application, accompanying documents and responses to the Secretariat queries. After careful consideration, we are pleased to inform you that the following decision was made by the HRCDC:

- The HRCDC has exercised its right under Regulation (8)(4)(b) and has made a **Conditional Declaration** that the public interest in carrying out the health research significantly outweighs the requirement of the Applicant(s) to seek explicit consent of the data subject, whose personal data is being processed for the above referenced health research study.

- The scope of the Conditional Declaration is for the following data processing activities specifically related to the above referenced health research study:

Scope of Declaration:
For the purpose of processing personal data of individuals that lack decision-making capacity where such processing involves accessing, collecting, pseudonymisation and analysis of data for the above reference study.

The scope of the declaration also covers the storage only of personal data for future, unspecified research studies but does not extend to the use or further processing of the personal data in other studies.

NOTE: The consent declaration is made for adult participants (over the age of 18 years) with an intellectual disability who lack the decision-making capacity to provide consent. The declaration does not cover the processing of personal data of children, as parental/legal guardian consent will be obtained for this participant cohort

- The following specific conditions have been attached to the Conditional Declaration as follows:

Condition 1. The Applicant is required to make all reasonable efforts to limit the risk of participant re-identification in publications and presentations in so far as is possible to do so. In addition, as an additional data security measure, the Applicant is requested to use a wholly separate unique participant study identifier during the dissemination of the study findings, that is

different to the identifier applied for the study and used during other study phases such as the collection and analysis stage.

Condition 2. Public and patient involvement (PPI) is considered an important activity by the HRCDC and is viewed as a key data protection safeguard in situations where the participant cannot provide consent. PPI also provides a valuable way of enhancing the level of transparency, which itself is an important data protection principle.

It is a condition of this declaration that PPI or engagement activities are undertaken with relevant individuals and groups for the reasons outlined above. Areas of consideration for PPI activity could include the development of the research, wider public transparency measures, the dissemination of research findings and the design, content and suitability of study information leaflets and assent/consent forms. Progress to meet this condition is a reporting requirement as part of the Annual Review.

Note for context: The HRCDC acknowledge that the study focuses on a rare form of genetic epilepsy. However, the HRCDC notes that there are relevant advocacy and representative organisations in the area of epilepsy and intellectual disability that can be engaged with on various elements of this study, for example Epilepsy Ireland.

Condition 3. As part of the assent/consent process the Applicant is requested to ensure that suitable processes are in place for the following:

(i) to identify the most appropriate individual who can provide proxy assent on behalf of the adult participant who lacks decision-making capacity, and who understands the will and preferences of the participant.
(ii) where proxy assent is being provided by the participant's Carer, it must be ensured that the Carer has good knowledge of the participant and understands their will and preferences.

Condition 4. The Applicant must ensure that the content, language and design of the study information leaflets, and assent/consent forms provided to adults with an intellectual disability are comprehensible and suitable for this cohort, and are tailored appropriately to adults with an intellectual disability. Specifically, the Applicant should:

(i) avoid using the same documents that have been designed for children when engaging with adults with an intellectual disability as their communication needs may differ.
(ii) engage with organisations, such as for example ACE Communication and Inclusion Ireland, that could assist with reviewing these documents to ensure suitably for adults with an intellectual disability. *(Please also see Condition 5)*

Note for context: The HRCDC was of the view that there should be no conflation of a child participant's intellectual ability with adults with an intellectual disability. Therefore, it is important not to assume that the content, language and design of study documents provided to a child is suitable for an adult with an intellectual disability.

Condition 5. The HRCDC requests that the study information leaflets, and assent/consent forms are further reviewed and amended to ensure clarity, transparency and consistency of information for participants and/or individuals providing assent. In this context the following observations were made by the HRCDC and should be addressed prior to the commencement of the study:

(i) it is noted that the study information leaflet contains duplicated information which should be removed,

(ii) clear information should be set out regarding withdrawing from the study, including what options are available with regards the personal data if proxy assent/consent is withdrawn and at what point in the study data cannot be deleted,
(iii) in addition to an email address, a phone number should be provided so that the participant or the proxy can contact the research team if they wish to discuss the study or exercise their data protection rights. Contact information must be provided in each of the study information leaflets used,
(iv) for adult participants who lack decision-making capacity, the terms 'proxy' and 'assent' should be used instead of 'legal guardian' and 'consent' when referring to or requesting agreement of the proxy to process the adult participant's personal data,
(v) the option for the storage of the data for future research provided in the participant consent form should state 'only if participant consent is obtained'. For the proxy assent form it should state 'only if participant consent/proxy assent is obtained'.

- The Declaration is made solely to the Applicant(s) who is the Data Controller and not to any other third party.

- The Declaration is made commencing 7th September 2021 and shall be valid until 30th September 2023 and for 7 years thereafter (until 30th September 2030) or upon confirmation that the personal data have been rendered anonymised or destroyed, or whichever occurs sooner.

In addition to the decision made by the HRCDC, the following standard conditions of the Declaration shall apply:

- the Applicant must complete an Annual Review to the HRCDC on the anniversary date of this decision letter and for every year, or part year, the Declaration is valid,

NOTE: Failure to submit an Annual Review to the HRCDC, a statutory requirement under the Health Research Regulations (Regulation 13(1)), may lead to a revocation of the consent declaration.

- the Applicant must have any necessary contractual obligations in place,
- all activities being carried out are in compliance with the General Data Protection Regulations, the Data Protection Act 2018 and Health Research Regulations 2018, for the duration of the Declaration,
- any breaches that occur that affect the integrity of the Declaration and the protection of data subjects, must be reported to the HRCDC,
- the health research must be conducted lawfully and ethically.

Lastly, the HRCDC have made further recommendations to the Applicant on the following areas of the application that should be considered by the Applicant. Note, these recommendations are not conditions attached, but nonetheless should be considered:

Recommendation 1. The Applicant is requested to consider whether the study information leaflet for the 10-18 year old cohort could be broken down into more age specific cohorts for example 10-13 year olds, 14-15 year olds, 16-17 year olds.

Note for context:
The HRCDC acknowledge that the consent declaration is not required for the processing of data of child participants younger than 18yrs, as Parent/legal guardian consent will be obtained, and

HRCDC
Health Research Consent Declaration Committee

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with assent from the child. However, the HRCDC discussed that the consent documents for the 10-18yr old group may be overly technical for the younger participants in this age bracket, and that these forms could be revised further and tailored for age appropriateness.

Recommendation 2. As part of the assent/consent process it is recommended that the Applicant ensures that functional capacity is re-assessed periodically during the course of the study and participant consent obtained.

Note for context: It should not be assumed that the functional capacity of an adult participant with an intellectual disability who lacks decision making capacity will not change during the lifetime of the study.

Please confirm acceptance of the Declaration within 30 working days of receipt of this letter, or the Conditional Declaration will lapse. Any clarifications required with respect to the decision made must be requested within the 30 day timeline.

Please notify your Data Protection Officer or equivalent authority within your organisation of this decision.

On behalf of the HRCDC and Secretariat, we wish you the very best of luck with the research study.

Kind regards,

Emily Vereker, PhD
Programme Manager, Secretariat
Health Research Consent Declaration Committee

Appendix 4

Everolimus (Afinitor) information sheet

How does everolimus work?

- Everolimus reduces excessive activity in an important cellular system in the brain and body, called the mTOR pathway
- The mTOR pathway is hyperactive in Tuberous Sclerosis
- Hyperactivity of this system causes seizures and growths in the brain, kidneys, lungs and skin

What are the indications for everolimus in tuberous sclerosis?

- Difficult-to-treat seizures
- In an important research study (the EXIST-3 trial), everolimus treatment significantly reduced seizures in almost half of patients with active epilepsy caused by tuberous sclerosis
- The response to everolimus increased over time
- Everolimus is also used to treat lumps in the brain, kidney and lungs in patients with tuberous sclerosis

Is everolimus used to treat other diseases?

- Everolimus may be used to prevent organ rejection in people with liver, kidney, lung or heart transplants
- Everolimus is also used to treat some cancers
- It is common for medications to be used for many different medical conditions

How do I take the treatment?

- Everolimus is taken once per day
- It is available in tablet or dispersible preparations
- It needs to be prescribed on High Tech Prescription by the neurology team

Will I need blood tests?

- Blood tests will be taken before starting treatment
- The everolimus level will be checked within 8 weeks of starting treatment or 8 weeks after a dose change

- Once the doctors are happy with the level/dose, the everolimus level is checked once per year

What are the side-effects?

- The most common side-effect is inflammation and/or ulcers of the mouth and lips (this is called stomatitis). Around 1 in 3 people taking everolimus experience this side-effect.
- It is important to maintain good oral hygiene when taking everolimus. We recommend using a soft toothbrush and children's toothpaste. We also recommend regular rinses with salt and water, particularly after eating. You should avoid spicy and acidic food when possible.
- If these measures don't help, there are additional mouthwashes we can prescribe.
- Other side-effects include chest infections and fevers. If you experience these it is important to contact us, as you may need to stop the everolimus for a period of time.
- Rarely, everolimus causes changes in the blood count and irregular periods in women.
- Women of reproductive age should use highly effective contraception as everolimus may be harmful to unborn babies.

If you have any questions or concerns please contact me
My email is patrickmoloney@rcsi.ie

References

1. French et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet.* 2016;388:2153-2163.
2. Davies et al. Management of everolimus-associated adverse events in patients with tuberous sclerosis complex: a practical guide. *Orphanet J Rare Dis*. 2017;12(1):35.
3. Novartis. Votubia (everolimus). Summary of Product characteristics. 2016. https://www.medicines.org.uk/emc/medicine/25054.

Appendix 5

Everolimus adverse events questionnaire (translated from German)

1. Infection
    - Fever
        - Duration (days)
        - Degree
    - C-reactive protein (CRP)
    - Antibiotic Therapy
        - Substance
        - Duration (days)
    - Infection focus
2. Ulcers/Stomatitis
    - Site
    - Treatment
    - Duration (days)
3. Gastrointestinal symptoms
    - Diarrhoea
    - Constipation
    - Vomiting
4. Upper respiratory tract symptoms
    - Rhinitis
    - Pharyngitis
    - Duration (days)
5. Menstrual cycle
    - Changes after initiation of everolimus
6. White blood cell count
    - Leukopenia
        - How severe
        - Duration
7. Lipids
    - Triglycerides
        - Level
        - Therapy needed
    - Cholesterol
        - Level
        - Therapy needed
8. Seizures
    - Seizure frequency worsening
    - Status epilepticus after initiation of everolimus

Side effects graded I-V, according to the Common Terminology Criteria for Adverse Events (CTCAE).

Appendix 6

Clinical Global Impression of Improvement (CGI-I)

Compared to the patient's clinical condition prior to medication initiation, the patient's condition is:

1. Very much improved since the initiation of treatment.
2. Much improved since the initiation of treatment.
3. Minimally improved since the initiation of treatment.
4. No change from baseline since the initiation of treatment.
5. Minimally worse since the initiation of treatment.
6. Much worse since the initiation of treatment.
7. Very much worse since the initiation of treatment.