Unravelling Molecular Genetic Causes and Disease Mechanisms in Landau Kleffner Syndrome

by

Adeline Ngoh

A thesis submitted to
University College London
for the degree of
DOCTOR OF PHILOSOPHY

I, Adeline Ngoh, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The cost of epilepsy to an individual lies not just in the burden of having recurrent seizures but also in the condition's neurodevelopmental, cognitive, psychological and social co-morbidities. Presently, our understanding of the pathophysiological mechanisms underlying epilepsy and its neurocognitive co-morbidities remains severely limited, translating to our current lack of targeted treatment options.

This PhD study aims to better understand the pathophysiological mechanisms underlying epilepsy and its neurocognitive co-morbidities through the clinical and molecular genetic study of a cohort of patients with Landau Kleffner syndrome (LKS), an epilepsy syndrome characterised by seizures, and neurodevelopmental regression in the form of loss of speech and language skills.

Patients were recruited from a database of children referred for LKS at Great Ormond Street Hospital's Developmental Epilepsy Clinic. Clinical data was extracted through case note review. As mutations in *GRIN2A,* a gene encoding the N2A subunit of the N-methyl-D-Aspartate (NMDA) receptor have previously been described in 8-20% of individuals with LKS and related disorders, recruited individuals were screened for *GRIN2A* mutations via Sanger Sequencing and multiplex-ligation probe amplification. Functional investigations exploring gene/protein expression, protein localisation and channel function were carried out on missense *GRIN2A* mutations identified. Individuals who screened negative for *GRIN2A* variants underwent whole exome sequencing or whole genome sequencing to identify novel genes associated with LKS.

This study has drawn conclusions that LKS is a neurodevelopmental disorder and clinical features influencing prognosis include age at onset of regression, non-verbal intelligence, and the presence of motor difficulties. *GRIN2A* mutations are likely to lead to LKS through overall NMDA receptor loss of function effects. Nonetheless, LKS may be a complex disorder with multi-factorial or oligogenic aetiology. Lastly, the long term potentiation pathway, important for learning and memory mechanisms, features strongly in the pathogenesis of LKS.

Impact Statement

Reflecting the rarity of Landau Kleffner Syndrome (LKS), this cohort of 91 patients, accumulated over nearly 30 years, is, to my knowledge, the largest cohort of LKS patients ever reported, with long follow-up durations affording an invaluable opportunity to explore comprehensively, the genotypic and phenotypic spectrum of this disorder.

Detailed endophenotyping of this cohort has enabled better characterization of LKS clinical features, facilitating rapid recognition of the disorder, accurate diagnosis and planning of services for each patient and family. The long follow-up duration of many paediatric patients into adulthood also furnishes the medical community with important information on disease evolution, treatment efficacy and prognosis. The identification of clinical features affecting long-term prognosis enables clinicians and families to design appropriate intervention. Lastly, and perhaps most importantly, the collection of data on preceding events, risk factors, treatment response, and disease course, provides clues to possible aetiology and pathophysiological mechanisms.

Mutations in the gene *GRIN2A* have previously been identified in 8-20% of individuals with LKS and related epilepsy aphasia spectrum disorders. The molecular genetic investigation of this LKS cohort substantiates the frequency of *GRIN2A* mutations in LKS, confirming that LKS is likely to be genetically heterogeneous. The functional investigations on *GRIN2A* mutations carried out in this study adds to a growing body of scientific evidence that *GRIN2A* mutations contribute to LKS pathogenesis through loss of function effects on N-methyl-D-aspartate receptors, offering the scientific community further ground in the unravelling of disease processes involved in LKS. Beyond *GRIN2A*, this study has identified through whole exome sequencing and whole genome sequencing studies, several other candidate genes which may contribute to LKS pathophysiology. Pending corroboration with other LKS cohorts, these offer further precious insight into pathophysiological mechanisms underlying LKS. Better understanding of pathophysiological mechanisms underlying this disorder is key to the design of more efficacious, targeted treatment options.

Neurodevelopment and neurocognition are crucially important to every child, family, and society; yet, our understanding of these pathways and the disease processes that disrupt them remains extremely limited. Whilst LKS is a rare neurodevelopmental disorder, the insights we gain through the study of this condition can have wider applications to many other developmental disorders affecting neurocognition, including other epileptic encephalopathies, and pervasive developmental disorders like autism spectrum disorder.

Acknowledgements

I would like to say a heartfelt thank you to Professor Manju Kurian. I could not have asked for a better mentor and primary supervisor. Professor Kurian has always touched me with her consideration and compassion towards each and every patient. Her passion for improving patient care through advancing scientific research has been inspirational. Professor Kurian's kindness and encouragement have supported me through some of my life's most challenging moments, and somehow, enabled me to complete this thesis despite these. I have learnt so much from Professor Kurian, and I could not be more grateful.

I would also like to extend my sincere gratitude to my secondary supervisors, Professor Robert Harvey and Professor Dimitri Kullmann. I am so fortunate to have been privy to guidance from such brilliant minds and I am very grateful for the invaluable insights both supervisors have given me throughout the course of this PhD. I am additionally grateful for Professor Harvey's generous provision of the prk-5-*GRIN2A* plasmid used for *GRIN2A* functional investigations during this PhD, for hosting me within his laboratory as I learnt in-situ mutagenesis, and for his team's collaboration with the *GRIN2A* electrophysiology experiments.

I would like to thank the late Professor Brian Neville, along with Dr Maria Clark and the Developmental Epilepsy Clinic (DEC) at Great Ormond Street Hospital (GOSH) for the collection and sharing of this Landau Kleffner Syndrome patient cohort. While I, unfortunately, only had limited time to work with Professor Brian Neville, I have been deeply moved by his work recognizing the importance of neurocognitive co-morbidities in epilepsy. During the course of this PhD study, I have greatly benefited from the expertise and kindness of Dr Maria Clark, who has taught me so much about Landau Kleffner Syndrome and neurodevelopment. The commitment of the DEC team to every patient is evident in the meticulous notes kept for each patient, and I am privileged to have had the opportunity to learn from these notes while collecting clinical data for this project's cohort study. I sincerely thank each and every member of DEC's multi-disciplinary team for their valued contribution to this project.

There have been so many other esteemed clinicians who have educated, inspired and supported me through the course of my PhD and Paediatric Neurology training. A special thank you to Professor Helen Cross whose dedication to her patients and to improving the care of epilepsy patients worldwide, has always been exemplary. Professor Cross has been an important source of support and motivation for me throughout my PhD and clinical training. I would also like to thank Dr Alasdair Parker, who has offered me much appreciated support for balancing clinical training with my research aspirations. I would also like to express my gratitude to Clinical Associate Professor Derrick Chan and the Paediatric Neurology team at KK Women's and Children's Hospital, Singapore for continuing to support me with this research and thesis, even after I started work in Singapore.

This project would not have been possible without my remarkable laboratory colleagues. I was fortunate to have had the opportunity to learn laboratory skills under the tutelage of Dr Esther Meyer. I am much obliged to Dr Katy Barwick for helping with Sanger verification of variants identified on whole exome sequencing and whole genome sequencing. I am also immensely grateful to Dr Amy McTague, Dr Natalie Trump, Dr Serena Barral, Dr Fatma Erdem, Dr Kimberley Reid, Dr Joanne Ng, Dr Apostolos Papandreou, Dr Karolin Kramer, Dr Lucia Abela, Dr Rachele Danti and Dr Dimitri Budinger, for unreservedly sharing various laboratory techniques with me, for their invaluable suggestions and ideas across our many discussions, and importantly, for dearly cherished friendships and memories.

I am thankful to have had collaborators that have added so much to this research study. A big thank you to: Dr Hywel Williams and the GOSgene bioinformatics team for facilitating bioinformatics and LUMPY analysis; the GOSH North East Thames Regional Genetics Service laboratory for their help with multiplex-ligation probe amplification investigations; Dr Dale Moulding (UCL GOS Institute of Child Health), for writing a macro that helped with immunocytochemistry analysis, Dr Maya Topf (UCL Birkbeck College) and Dr Sony Malhotra (University of Cambridge) for their work with structural homology modelling; and Professor Robert Harvey, Dr Joseph Lynch and Dr Xiumin Chen (Sunshine Coast University, Australia) for carrying out the electrophysiology experiments.

I gratefully acknowledge Action Medical Research, Guarantors of Brain and Friends of Landau Kleffner Syndrome for so generously funding this study.

I pay a sincere tribute to all Landau Kleffner Syndrome individuals and their families. It has been an incredible honour to work alongside such valour and perseverance.

My greatest blessing has been my wonderful husband, Hon Shing, my mother and my family. I could not possibly do anything without their unwavering love and encouragement.

Last but not least, I thank my late father, Robert Ngoh, who has been my life-long inspiration with his words: "*whatever talents you may have – be they great or small, do not laze; do your best with all you have, to serve those in need".* This thesis is dedicated to his memory.

Contents

List of Figures

List of Tables

1 Chapter 1: Introduction

1.1 Epilepsy and its Co-Morbidities

The International League Against Epilepsy (ILAE) defines epilepsy as "a disease characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition".

This definition clearly drives home the fact that, clinicians managing childhood epilepsy face challenges that encompass more than just seizure control.

In addition to seizures, as many as 20-25% of children with epilepsy have cognitive impairment, severe enough to fall within the intellectual disability range (Berg, 2011). Indeed, even when intelligence quotients fall within the normal range, neuropsychological testing often reveals selective difficulties in various domains, including attention, memory, and executive function. Furthermore, children with epilepsy often have difficulties with language, motor function and/or behaviour.

The importance of appropriately managing these difficulties alongside seizure control cannot be over-stated, as these have significant impact on a child's education, and both the child's and family's quality of life.

Attempts to prevent, treat, or even reduce the impact of these co-morbidities, however, can only be successful if pre-dated by adequate understanding of the neurobiological mechanisms underlying neurocognitive difficulties in epilepsy.

This introductory chapter reviews what we currently know about the neurobiological mechanisms that influence neuro-cognitive deficits in epilepsy, and introduces Landau Kleffner Syndrome, an epilepsy syndrome where neurocognitive difficulties, particularly in the domain of speech and language, typically outweigh the burden of clinical seizures.

The subsequent chapters (2-7) then outline the clinical and molecular genetic investigation of a cohort of individuals with Landau Kleffner Syndrome; and discuss how these studies may contribute to our current knowledge of this syndrome, and our understanding of the pathways leading to neurocognitive deficits in epilepsy.

1.2 Key Physiological Mechanisms in Learning and Memory

1.2.1 Historical Perspectives

Elucidation of the molecular, cellular and network mechanisms governing learning and memory, has long been a major goal within neuroscience. Whilst our knowledge remains incomplete, significant strides have been made in various domains of neuroscience, in the last century, that have allowed us to start piecing this complex puzzle together.

The concept that information is stored in the brain as a result of alternations in synaptic efficiency, first arose in the late nineteenth century. At this time, Santiago Ramon y Cajal, considered by many to be the "father of Neuroscience", first demonstrated that neuronal networks do not exist in cytoplasmic continuity, but rather communicate with each other by means of specialized junctions, called synapses (Cajal, 1911). Cajal proposed that rather than forming new neurons, the mechanism of learning and memory involved strengthening the connections between existing neurons to improve the efficacy of their communication.

Approximately half a century later, in the late 1940s, a Canadian psychologist, Donald Hebb refined these ideas, proposing a coincidence-detection rule, in which the synapse linking two neuronal cells is strengthened if both cells are triggered simultaneously. Hebb proposed: "*when cell A excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased"* (Hebb, 1949). This concept later came to be known as Hebbian plasticity. The capacity for activity-dependent changes in neuronal connectivity became referred to as neuro-plasticity.

Although the concept of neuroplasticity provided an elegant model for learning and memory formation, experimental evidence for its existence was initially difficult to find. In hindsight, this may have been because early studies investigated peripheral nervous system synapses, which are considered to be relatively non-plastic (Sweatt, 2016).

In the 1950s, the profound memory loss observed in a patient, H.M., after bilateral medial temporal lobe resection for psychosis and intractable seizures (Scoville and

Milner, 1957) prompted the medical community to focus attention on the hippocampus as an important brain region for memory formation.

In the late 1960s and early 1970s, perhaps facilitated by development of *in vivo* electrophysiological techniques, the first experimental evidence of Hebbian plasticity emerged in the laboratory of Per Anderson in Oslo (Sweatt, 2016). Building on work that Anderson's student, Terje Lomo did as part of his PhD, Timothy Bliss, a Psychology major and Lomo collaborated on a series of experiments based on *in vivo* recordings of synaptic responses in the dentate gyrus of rabbits (Bliss and Lomo, 1973). Investigating perforant path inputs from the entorhinal cortex onto dentate granule cells, they discovered that delivery of brief trains of high frequency stimulation triggered a rapid and sustained increase in the efficiency of synaptic transmission within selective circuits. They also demonstrated that the probability of a post-synaptic neuron firing an action-potential could be increased by a constant level of pre-synaptic stimulation. In their landmark paper in 1973, they labelled this phenomenon "long term potentiation" (LTP). Bliss and Lomo's landmark paper has provided a strong basis for several more discoveries that have helped us to advance our knowledge on how we learn, remember and modify our behaviour.

We now know that there are several different forms of LTP, that LTP is complemented by an antagonistic process known as long term depression (LTD), and that LTP can involve both Hebbian and non-Hebbian mechanisms.

Whilst LTP occurs at synapses throughout the brain, the type of LTP that occurs varies, depending on factors, including the region of the brain involved and developmental age. For example, LTP at synapses between dentate gyrus mossy fibres and Cornu Ammonis area (CA) 3 pyramidal cells occurs through different mechanisms from LTP at CA1 hippocampal synapses (Nicoll, 2017). In addition, the molecular mechanisms for LTP in an immature hippocampus differ from those in an adult hippocampus (Swann et al., 1990).

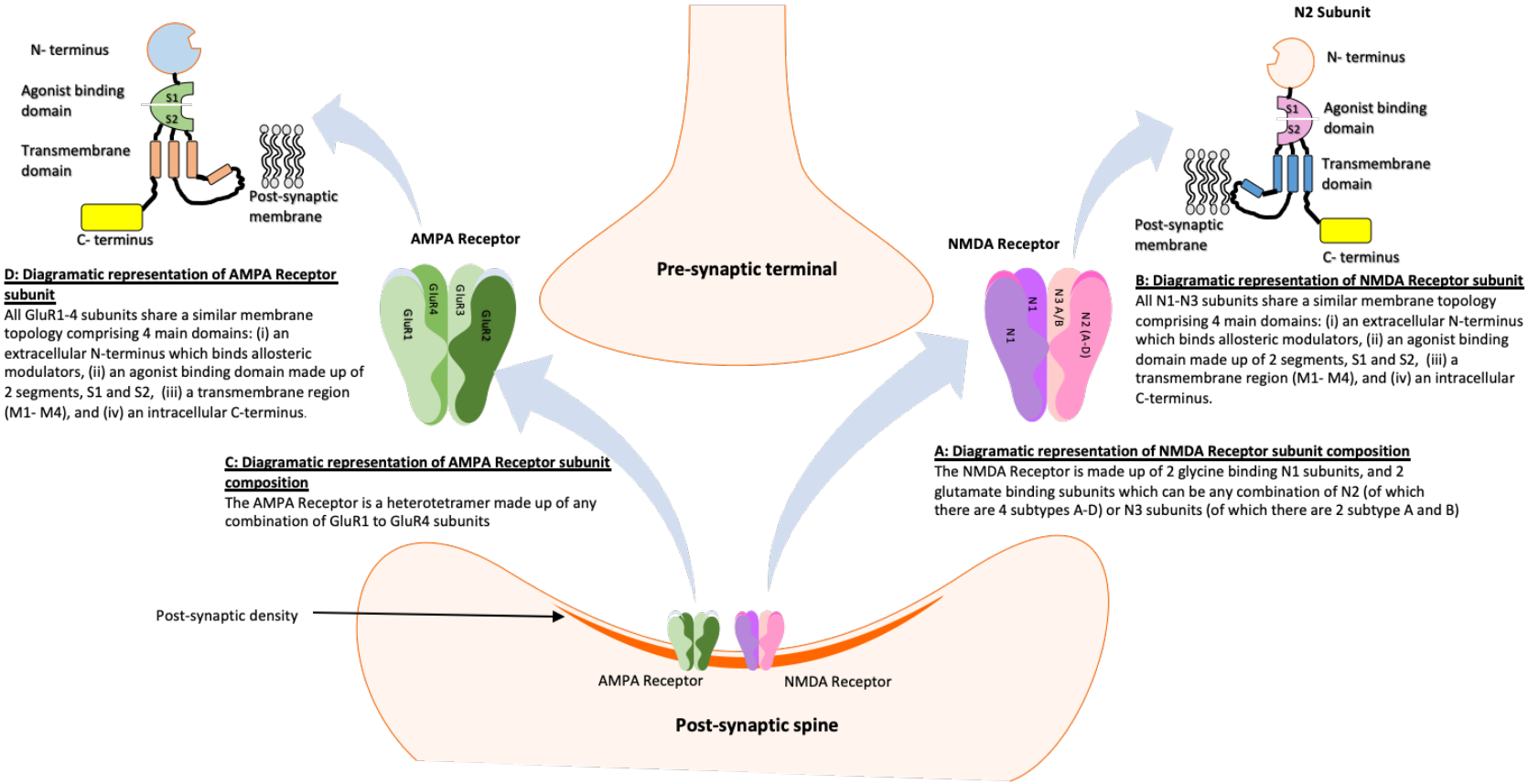
1.2.2 Long Term Potentiation

Of the various different forms of LTP, N-methyl-D-aspartate (NMDA) receptor-mediated LTP in the adult CA1 hippocampus, is the most widely studied. As such, this is often viewed as the "prototypical" form of LTP.

1.2.2.1 The Glutamatergic synapse

The glutamatergic synapse in the CA1 hippocampus comprises the pre-synaptic and the post-synaptic dendritic spine (**Figure 1-1**). Spines are chemically and electrically segregated small micro-compartments that appear as protrusions on the dendrites of neurons. The post-synaptic density (PSD) is a specialized area just beneath the membrane of the post-synaptic spine. It lies in close apposition to the pre-synaptic terminal and contains within it, several proteins that are crucial for synaptic signal transduction including glutamate receptors, scaffolding proteins and cell-signaling molecules (Okabe, 2007).

Figure 1-1 Schematic Representation of a Glutamatergic Synapse and NMDA/AMPA Receptors



D: Diagramatic representation of AMPA Receptor subunit
All GluR1-4 subunits share a similar membrane topology comprising 4 main domains: (i) an extracellular N-terminus which binds allosteric modulators, (ii) an agonist binding domain made up of 2 segments, S1 and S2, (iii) a transmembrane region (M1- M4), and (iv) an intracellular C-terminus.

B: Diagramatic representation of NMDA Receptor subunit
All N1-N3 subunits share a similar membrane topology comprising 4 main domains: (i) an extracellular N-terminus which binds allosteric modulators, (ii) an agonist binding domain made up of 2 segments, S1 and S2, (iii) a transmembrane region (M1- M4), and (iv) an intracellular C-terminus.

C: Diagramatic representation of AMPA Receptor subunit composition
The AMPA Receptor is a heterotetramer made up of any combination of GluR1 to GluR4 subunits

A: Diagramatic representation of NMDA Receptor subunit composition
The NMDA Receptor is made up of 2 glycine binding N1 subunits, and 2 glutamate binding subunits which can be any combination of N2 (of which there are 4 subtypes A-D) or N3 subunits (of which there are 2 subtype A and B)

1.2.2.2 The NMDA Receptor

The NMDA receptor is a heterotetrameric voltage-gated ionotropic receptor (**Figure 1-1**). It is made up of 2 glycine binding N1 subunits (of which there are 8 different subtypes generated by alternative splicing from a single gene), and 2 glutamate binding subunits, which can be any combination of N2 and N3 subunits. There are 4 different types of N2 subunits (A-D), and 2 different types of N3 subunit (A and B). Six separate genes encode the different N2 and N3 subtypes, and each N2 and N3 subtype has different pharmacological properties. All N1-N3 subunits share a similar membrane topology comprising 4 main domains: (i) an extracellular N-terminus which binds allosteric modulators, (ii) an agonist binding domain made up of 2 segments, S1 and S2, (iii) a transmembrane region, and (iv) an intracellular C-terminus.

1.2.2.3 The AMPA Receptors

The NMDA receptors' role in facilitating LTP is complemented by another class of ionotropic glutamate receptors, the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors **(Figure 1-1).** AMPA receptors are the most commonly found receptors in the central nervous system. They are also heterotetramers, which are composed of different combinations of 4 types of subunits, GluR1 to GluR4, each encoded by a single gene. Like the NMDA receptor subunits, each subunit has a large extracellular domain, a ligand binding domain 4 trans-membrane domains and a cytoplasmic tail. AMPA receptors open rapidly in response to glutamate binding and close rapidly. They are thus responsible for fast excitatory synaptic transmission.

1.2.2.4 LTP Induction

Unlike AMPA receptors, NMDA receptors require more than just glutamate and glycine binding for activation. The NMDA receptor's properties as both a ligand- gated and voltage-gated ion channel enable it to serve as an ideal "coincidence detector" to facilitate Hebbian plasticity (Sweatt, 2016). In addition to glutamate and glycine binding to the receptor, the simultaneous depolarization of the post-synaptic membrane is also required to activate the NMDA receptor ion-channel. This is because at resting membrane potential, there is a voltage-dependent magnesium (Mg^{2+}) block at the pore of the NMDA receptor ion channel (**Figure 1-2**).

When the post-synaptic membrane is at resting potential and glutamate is released by a pre-synaptic nerve terminal in response to an excitatory potential, AMPA receptors are activated, but not, initially, NMDA receptors (**Figure 1-2**). The influx of sodium ions through AMPA receptor channels starts to depolarize the post-synaptic membrane. If the pre-synaptic stimulus is strong or frequent enough to lead to sufficient depolarization of the post-synaptic membrane, the voltage-dependent Mg^{2+} block on the NMDA receptor ion channel is removed which results in the NMDA receptor being activated, allowing calcium ions (Ca^{2+}) to flow through the channel into the post-synaptic cell. It is this calcium influx that triggers a series of events leading to the augmentation of the efficacy of the synapse involved – key to the phenomenon of LTP.

The series of events leading to LTP (simultaneous glutamate-binding and sufficient membrane depolarization) is termed LTP induction. The series of events that occur after LTP is induced is known as LTP expression. LTP-expression can be divided into an early-phase (E-LTP), occurring within 60 minutes post induction and a late phase (L-LTP), occurring more than 60 minutes post induction (Bliss and Cooke, 2011).

1.2.2.5 LTP Expression- Early-LTP

The molecular events that occur during LTP expression are not completely understood with a number of contentious issues. Some hypotheses are illustrated in **Figure 1-2.**

In summary, during LTP induction, NMDA receptor activation results in calcium influx into the post-synaptic terminal. Ca^{2+} binds to the secondary messenger protein Ca^{2+}/Calmodulin. This, in turn, activates protein kinases, such as calcium-calmodulin dependent kinase 2 (CaMKII), which in turn phosphorylate various proteins. Three important processes that result from this are: (i) increased AMPA receptor channel conductance; (ii) increased expression of AMPA receptors at the post-synaptic membrane through exocytosis; and (iii) enlargement of the post-synaptic dendritic spine, thus augmenting synapse efficacy.

In addition to post-synaptic changes, there is also some evidence to suggest that there is augmentation of pre-synaptic glutamate release during LTP-expression. It is not clear how this happens but the process may involve retrograde messengers such as nitric oxide or arachidonic acid. It has also been proposed that this may occur through adhesion molecules binding the pre-synaptic and post-synaptic membranes (Abraham and Williams, 2003).

1.2.2.6 Late-LTP

Unlike E-LTP, which only involves the enlargement of the post-synaptic dendritic spine, L-LTP involves the enlargement of the entire synapse. In L-LTP, the persistence of kinase activity triggered in E-LTP, initiates a cascade of cell-signalling events culminating in changes in gene-expression and the synthesis of new proteins. This is partly evidenced by the fact that blocking gene transcription, either through application of the transcription-inhibitor actinomycin D, or through the inhibition of various transcription factors, blocks L-LTP and disrupts long term memory (Sweatt, 2016). Again, the mechanisms that mediate new protein synthesis are not completely understood. However, the cyclic-adenosine monophosphate (cAMP) dependent signalling pathway, involving cAMP dependent kinase (PKA), mitogen activated protein kinases (MAPK), and transcription factors such as cyclic-AMP responsive element binding protein (CREB), is likely to play an important role **(Figure 1-2).**

Of note, other neurotransmitters including dopamine, noradrenaline, serotonin and acetylcholine have a modulatory role in L-LTP. Through their respective receptors,

they activate the cyclic-AMP dependent signalling pathway. This may be the mechanism through which reactions such as reward, punishment, arousal and attention modify learning and memory (Bliss and Cooke, 2011). This is also one example of a non-Hebbian mechanism in LTP. A Hebbian rule is a two-factor rule – involving the contemporaneous occurrence of glutamate binding to NMDA receptors and post-synaptic depolarization. In this regard, L-LTP has been termed "neo-Hebbian", as it also involves other neurotransmitters as a third modulatory factor (Lisman, 2017).

We have yet to establish the identities of all the proteins synthesized during L-LTP. Some proteins that have been identified to have increased expression during LTP include: (i) receptor subunits – N2A and N2B NMDA receptor subunits, and the GluR1 subunit of the AMPA receptor; (ii) receptor associated proteins such as HOMER1 which regulates group 1 metabotropic glutamate receptor function; and (iii) structural proteins such as activity regulated cytoskeletal protein (ARC), and microtubule associated protein 2 (MAP2) (Abraham and Williams, 2003, Sweatt, 2016).

Messenger ribonucleic acid (mRNA) and ribosomes are located at synaptic regions so protein translation can be stimulated locally within the dendritic spines in response to LTP, bringing about rapid modification of the synaptic proteome. However, new protein synthesis that is dependent on gene transcription needs to take place in the soma. It has been proposed that, in this case, newly translated proteins are incorporated specifically to the synapse undergoing plasticity and not neighbouring synapses through an incompletely understood process, termed synaptic tagging (Viola et al., 2014).

1.2.3 Long term depression

A complementary process to LTP, long term depression (LTD) results in a reduction in the efficacy of synaptic transmission. Heterosynaptic LTD occurs in a synapse when neighbouring synapses are strongly stimulated. Homosynaptic LTD occurs when there is a long period of low-frequency stimulation of a particular synapse (Lisman, 2017). Like LTP, LTD occurs in most excitatory synapses throughout the brain, allowing for

the establishment of bi-directional synaptic plasticity (Pinar et al., 2017). Furthermore, like LTP, there are different forms of LTD occurring at different regions of the brain. Within the CA1 region of the hippocampus, the induction of LTD depends on NMDA receptor activation. When the NMDA receptor is activated and Ca^{2+} ions permeate the post-synaptic membrane, the concentration and temporal profile of the Ca2+ determines if LTP or LTD is triggered (Bliss and Cooke, 2011). Whilst brief and steep Ca^{2+} transients induce LTP through the activation of protein kinases, prolonged, low Ca^{2+} transients induce LTD through the activation of calcium-dependent phosphatases such as calcineurin and protein phosphatase 1. These dephosphorylate their target proteins and generally have opposite effects to protein kinases activated in long term potentiation (Bliss and Cooke, 2011).

Figure 1-2 LTP Induction and Expression

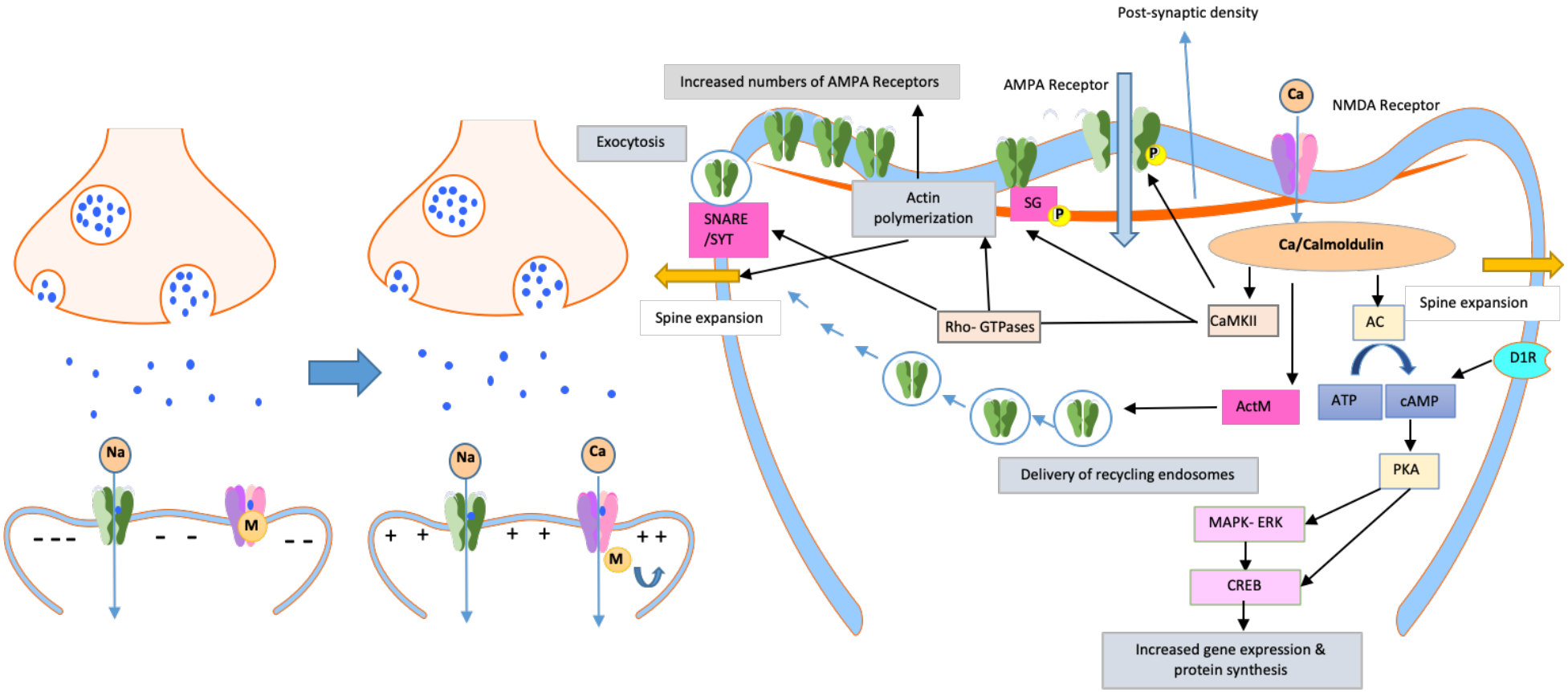


Figure 1-2 LTP Induction and Expression Legend

Long Term Potentiation Induction

When the post-synaptic membrane is at resting potential and glutamate is released by a pre-synaptic nerve terminal, only AMPA receptors are initially activated. AMPA receptor activation depolarizes the post-synaptic membrane, thereby removing the voltage-dependent Mg^{2+} block on the NMDA receptor channel allowing receptor activation and Ca^{2+} influx.

Long Term Potentiation Expression – E-LTP

When NMDA receptors are activated, there is influx of calcium into the cell. Calcium binds to Ca^{2+}/Calmodulin which activates protein kinases including **CAMKII**. This results in:-

(1) Increased AMPA receptor channel conductance – CAMKII phosphorylation **(P)** of the C-terminal tails of the GluR1 subunit of AMPA receptors increases their channel conductance

(2) Increased number of AMPA receptors. This may happen through:

(a *Post-synaptic density (PSD)-centric model* – AMPA receptors located on the peri-synaptic membrane freely diffuse in and out of synapses. During E-LTP, AMPA receptors are translocated from the extra-synaptic membrane and anchored into slots within the PSD. This may happen as a result of: (i) CAMKII activation of **Rho GTPases**, such as **CDC42** and **RhoA**. Rho GTPases inhibit the negative actin polymerization regulators actin depolymerizing factor (ADF) and cofilin, thereby leading to actin polymerization. Actin polymerization may lead to rearrangement of PSD scaffolding molecules, increasing the number of slots available for AMPA receptor anchoring; and/or (ii) CAMKII phosphorylation of **stargazin (SG),** a transmembrane AMPA receptor protein (TARP) present in the PSD, thereby increasing AMPA receptor diffusion into the PSD (Herring and Nicoll, 2016, Lisman, 2017), Lisman, 2017).

(b*) Vesicle-centric model* – There is increased exocytosis of AMPA receptors onto the post-synaptic membrane. Whilst at basal conditions constitutive recycling processes traffic GluR2 and GluR3 subunits to the post-synaptic membrane, during LTP, GluR1 subunit containing AMPA receptors are rapidly inserted into the post-synaptic membrane. Mechanisms for this are unclear but may involve increased delivery of recycling endosomes for exocytosis by **actin motors (ActM)** which activate on Ca^{2+}/Calmodulin binding (Wang et al., 2008). Exocytosis into the post-synaptic membrane is then mediated by **synatotagmins (SYT)** and **SNAREs** (soluble NSF(N-ethylmaleimide-sensitive factor) attachment protein receptors) (Rizo et al., 2006, Wu et al., 2017). There is also some evidence that Rho GTPases activated by CAMKII can directly interact with SNAREs and promote vesicular exocytosis (Nevins and Thurmond, 2005).

(3) Enlargement of the post-synaptic spine: in addition to rearrangement of PSD scaffolding molecules and increasing the number of slots available for AMPA receptor anchoring. Rho-GTPase induced actin polymerization also results in expansion of the actin cytoskeletal network resulting in dendritic spine enlargement (Herring and Nicoll, 2016).

Long Term Potentiation Expression – L-LTP

(1) Calcium influx activates **adenyl cyclase (AC),** which catalyses the conversion of adenosine triphosphate (ATP) to cyclic-AMP (cAMP). Other neurotransmitter receptors such as **dopamine receptors (D1R)** also modulate LTP via their action on AC.

(2) This activates **cAMP dependent kinase (PKA),** which phosphorylates the **extracellular signal regulated kinase (ERK)** subfamily, of **mitogen activated protein kinases (MAPK**)

(3) ERK phosphorylates **cAMP dependent response element binding protein (CREB**). PKA also phosphorylates this directly.

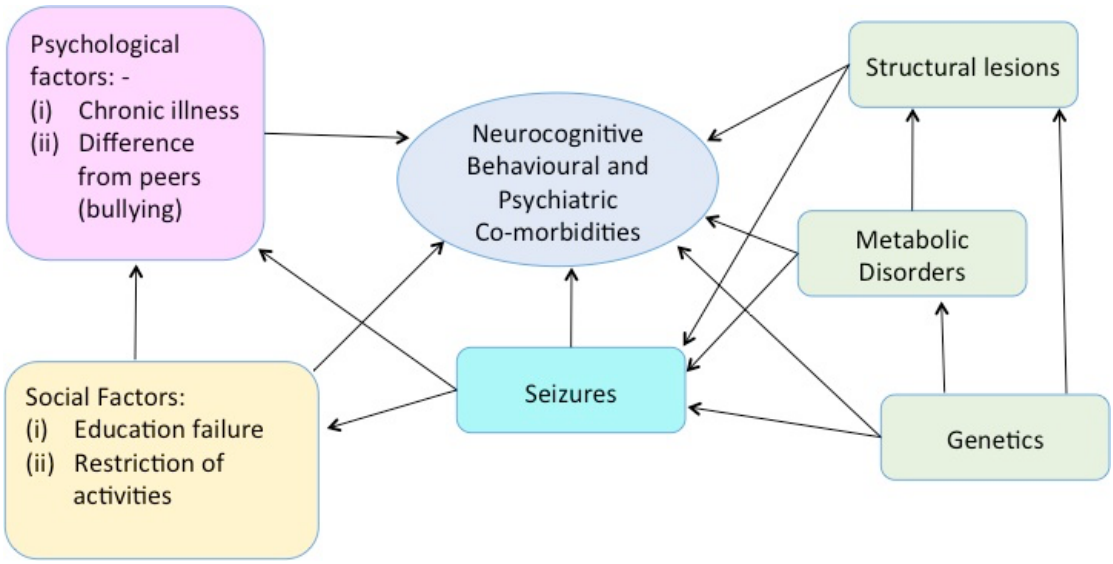
(4) CREB forms homodimers with other members of the **B-Zip** family of transcription factors. This complex binds to the promoter region of responsive genes stimulating their expression (Abraham and Williams, 2003).

1.3 Neurocognitive Deficits in Epilepsy: Proposed Mechanisms

The biopsychosocial model, commonly used in the field of Neuropsychiatry, can be used to consider the multifactorial aetiologies underlying the cognitive, behavioural and psychiatric co-morbidities associated with epilepsy (**Figure 1-3**).

Whilst all these factors are important to consider, better understanding of causal neurobiological mechanisms necessitates further examination of: (i) common/shared causative factors (factors that can lead to both epilepsy and cognitive/behavioural disorders); and (ii) the impact of recurrent seizures and/or abnormal electrographic activity on brain development and function.

Figure 1-3 Biopsychosocial model – co-morbidities associated with seizures



1.3.1 Common causative factors

The basic pathophysiology of seizures involves an imbalance between excitatory and inhibitory activity in the brain. In addition to its role in learning and memory, the LTP pathway discussed has also been implicated in epileptogenesis through the generation of hyperexcitability (Bliss and Cooke, 2011).

In essence, processes involved in neurocognition and the regulation of the balance between excitatory and inhibitory neural activity, share a common dependence on the

integrity of brain structure and function. As such, any condition that impacts on brain structure or function can potentially result in both seizures and neurocognitive deficits.

Such conditions may be broadly classified into: (i) structural brain lesions, which may be congenital, inherited or acquired; (ii) metabolic disorders; and (iii) genetic diseases (**Figure 1-3**). It is important to note that within this crude classification system, there is significant overlap. For example, nearly all metabolic disorders are inherited/genetic and some are also associated with structural malformations, as seen in peroxisomal disorders, mitochondrial diseases and congenital disorders of glycosylation.

Approximately 25% of childhood epilepsy is associated with identifiable structural brain lesions evident on brain magnetic resonance imaging (Berg, 2011). Structural lesions can impact cortical excitability and are often associated with aberrant neuronal connections, thereby increasing the risk of epilepsy (Shafi et al., 2015). Neurocognitive impairment associated with such structural lesions is often related to the extent of the lesion, the specific brain region involved, and, for acquired lesions, the age when the injury was sustained (Berg, 2011). Metabolic disorders typically result in the accumulation of toxic metabolites, and/or failure to produce sufficient energy or key proteins, metabolites or neurotransmitters required for normal brain function. Such impairment of neuronal cell metabolism may then lead to dysregulation of cortical excitability, resulting in both seizure susceptibility and neurocognitive impairment (Sharma and Prasad, 2017).

It is now well recognized that genetic influences constitute a major factor in both childhood epilepsy and neurocognitive impairment. Indeed, in the paediatric population, it is estimated that approximately 40-60% of epilepsies have an underlying genetic aetiology (Zuberi and Symonds, 2015). It is also increasingly clear that genetic variation can concurrently lead to both epilepsy and cognitive impairment (Heyne et al., 2018).

Since the completion of the human genome project in 2003, impressive advances in molecular genetic technologies have led to the rapid evolution of an ever-growing compendium of genes linked to childhood epilepsy syndromes. This is discussed in further detail in Section 1.4.

1.3.2 Impact of recurrent seizures and abnormal electrographic activity on brain development and cognitive outcome

Regardless of underlying aetiology, it is proposed that epileptic activity itself (both manifest seizures and subclinical epileptic discharges) can contribute to neurocognitive impairment. This concept has arisen from various observations both in clinical studies and within the laboratory.

1.3.2.1 Clinical Observations

Dr William James West first described a case of West syndrome in 1841. William Landau and Frank Kleffner reported the clinical features of Landau Kleffner Syndrome in 1957. William Lennox and Henri Gastaut described Lennox-Gastaut syndrome in the 1960s. In the 1970s, Carlo Alberto Tassinari described electrical status epilepticus in sleep (ESES), the electroencephalographic signature associated with the syndrome, epilepsy with continuous spike waves during sleep (ECSWS). It is striking that all these epilepsy syndromes have in common, the association of profound neurocognitive decline, with significantly abnormal electroencephalograms (EEG), even inter-ictally.

The early historical descriptions of these electroclinical syndromes has fuelled the belief that epileptic activity itself can lead to neurocognitive impairment (Capovilla et al., 2013). This concept is supported by various other clinical observations. One example is the notion that whilst syndromes, such as Lennox-Gastaut syndrome, can arise from a variety of different genetic, or structural aetiologies, patients tend to have a similar neurocognitive outcome (Chapman et al., 2015); this, to some, suggests that a common final pathway (possibly related to seizure activity) may be responsible for poor neuro-cognitive outcome.

Another clinical observation that lends some support to the concept that epileptic activity leads directly to cognitive impairment, is the repeated finding in various studies, that regardless of aetiology or type of epilepsy, younger age at seizure onset is associated with worse neurocognitive outcomes. It has been postulated that epileptic activity during critical periods of early development may interfere with the brain's efforts to establish functional connections and key neuronal networks (Berg, 2011).

Perhaps the most convincing argument for epileptic activity driving cognitive deficit comes from studies reporting neurocognitive rescue after effective resolution of seizure activity. In West syndrome, even when structural aetiology is identified, early control of clinical seizures and hypsarrhythmia on EEG improves neurocognitive outcome (O'Callaghan et al., 2011, Humphrey et al., 2014). Furthermore, several studies have demonstrated that early epilepsy surgery can improve neurocognitive and behavioural outcome (Berg, 2011, Engel et al., 2012, Dwivedi et al., 2017). For the most part, it appears that cognitive improvement may be dependent on a reduction in seizure burden (Berg, 2011).

1.3.2.2 Within the laboratory - Animal models of epilepsy and learning

Within the laboratory, electrophysiological and histochemical studies have been coupled with animal behavioural studies to study the effects of epileptic activity on learning and memory.

McCabe et al (2001) demonstrated that recurrent seizures in developing rats adversely affected neurogenesis. Rats that were subject to 25-fluorothyl induced seizures in the first 5 days of life had a significant reduction in the number of newly developed neurons within the dentate gyrus and hilus, when compared to control animals. This reduction in neurogenesis was observed for up to 6 days following the last seizure.

In addition, recurrent seizures in developing rats (postnatal day 6 to postnatal day 10) have also been shown to lead to dendritic spinal loss in CA3 pyramidal cells (Jiang et al., 1998), and alterations in excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA) neurotransmitter receptor subunit expression (Bo et al., 2003, Ni et al., 2004).

Meldrum et al. demonstrated that electrographic seizures, even in the absence of associated behavioural change can result in brain damage. In their experiment, adolescent baboons that were paralyzed and artificially ventilated, with maintenance of normal blood pressure and blood glucose concentration, developed ischaemic brain damage when subjected to electrographic seizures via intravenous injection of bicuculline (Meldrum et al., 1973).

Animal models of neurocognition commonly employ the Morris water-maze to test hippocampal dependent spatial memory (Vorhees and Williams, 2006). Using this technique, several studies have consistently shown that animals subject to recurrent early seizures have visuo-spatial memory impairment (Holmes, 2009). Neill et al. have also shown that recurrent early seizures have a negative impact on auditory discrimination (Neill et al., 1996). Holmes et al investigated the firing patterns of hippocampal neurons in freely moving rats. They observed that certain cells fire selectively when a rat moves through a particular location in space. These cells are known as place cells. Compared with control rats, they demonstrated that rats subject to recurrent seizures had significant deficits in the coherence and stability of place cell action potentials (Holmes, 2009).

As such, although it is likely that cognitive deficits and epilepsy co-occur due to dependence on common developmental/biological pathways, there is also ample evidence to suggest that epileptic activity, itself, can bring about cognitive impairment.

1.3.2.3 Evolving Definitions of "epileptic encephalopathy"

.The concept that seizure activity can result in neuro-cognitive impairment was first included in the International League Against Epilepsy (ILAE) classification proposal in 2001 (Engel, 2001). Herein, the term "epileptic encephalopathy" was used to refer to conditions in which "the epileptiform abnormalities themselves are believed to contribute to progressive disturbance in cerebral function".

In the 2006 ILAE Classification report, epileptic encephalopathy was defined as a group of conditions that involved epilepsy-dependent neurodegenerative processes, instead of metabolic or encephalitic processes (Engel, 2006). Subsequently, in the 2009 ILAE report on epilepsy classification and terminology, the definition of epileptic encephalopathy was refined to where "the epileptic activity itself contributes to cognitive and behavioural impairment *above and beyond* what might be expected from the underlying pathology alone" (Berg et al., 2010). As such, while the term "encephalopathy" refers broadly to brain disorders where there is neurocognitive decline due to heterogeneous causes (including hypoxic-ischaemic, metabolic, autoimmune, infectious, and toxic), the term "*epileptic* encephalopathy" was reserved

for conditions in which seizure activity is considered to be significantly contributory towards the observed neurocognitive impairment.

The term “epileptic encephalopathy” therefore creates some controversy, as often for a given epilepsy syndrome, it can be difficult to decipher exactly how much of the observed neurocognitive deficit is due to seizure activity, and how much is due to the actual underlying aetiology. This is particularly topical in recent years, where genetic aetiologies have emerged for conditions that we have traditionally viewed as “epileptic encephalopathies”, but where we have now come to learn that the causative gene not only increases seizure susceptibility, but also has independent pathophysiological effects on neurodevelopmental processes, thereby impacting cognition (Section 1.4). Nonetheless, this distinction is important because the fundamental ethical principle of non-maleficence warrants us to “first do no harm”. Whilst some studies suggest that resolution of seizure activity can improve cognitive outcomes, erroneously ascribing neurocognitive deficit purely to seizure activity when it may also be attributed to underlying aetiology, could potentially result in unjustified over-treatment. Considering that anti-epileptic drugs can also often have negative effects on neurocognition, this can lead to a damaging vicious cycle, further affecting cognitive outcome (Capovilla et al., 2013).

In the latest 2017 revision of epilepsy classifications and terminology, to lessen the confusion surrounding the term “epileptic encephalopathy” and in recognition of the fact that many childhood epilepsy syndromes are associated with genetically determined developmental syndromes, it was proposed that we use the terms “developmental and/or epileptic encephalopathy”. Based on what is judged to be the impact of developmental processes versus epileptic processes on cognitive burden, a condition may be referred to as a “developmental encephalopathy”, “epileptic encephalopathy” or a “developmental and epileptic encephalopathy” (Scheffer et al., 2017).

1.4 Genetics and Epilepsy

"It's a history book - a narrative of the journey of our species through time. It's a shop manual, with an incredibly detailed blueprint for building every human cell. And it's a

transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent and cure disease." *Francis Collins, Director of National Human Genome Research Institute, describing the genome (2000)*

1.4.1 History and basic principles

Through his experiments on pea plants, Gregor Johan Mendel, an Augustinian friar and scientist is credited with the first recognition that phenotypic traits are transmitted from parent to offspring through discrete, invisible "factors", now known as "genes". Mendel published his work in 1866, describing 3 important principles of genetics – the law of segregation, the law of independent assortment and the law of dominance. These have since come to be known as Mendel's laws of inheritance.

In the early 20th century, building on Mendel's discoveries, Thomas Hunt Morgan, an evolutionary biologist, took our understanding of the principles of genetics one leap further through his work on the fruit-fly, *Drosophila melanogaster*. Morgan discovered that genes were located on chromosomes after noting the X-linked inheritance of a mutant white-eye gene. Through learning that other traits also located on the X-chromosome may or may not sort independently from the white-eye gene, Morgan uncovered the principles of genetic- linkage and recombination.

Morgan received a Nobel Prize for Physiology or Medicine in 1933 for essentially uncovering the chromosomal basis of heredity. Since then, several important advancements following Morgan's work have enabled us to get to where we are today. In 1953, Francis Crick and James Watson discovered the molecular basis of heredity through identification of the deoxyribonucleic acid (DNA) double helix. This discovery also earned them a Nobel Prize in 1962. In the 1960s, key studies elucidated mechanisms of DNA replication, transcription and protein translation. Marshall Nirenberg shared a Nobel Prize in 1968 with Har Gobind Khorana and Robert Holley for their contribution to our knowledge of these mechanisms. Armed with the knowledge gained in the previous decade, the invention of Sanger sequencing and recombinant DNA technologies in the 1970s, have provided scientists with various means to manipulate genes, and thereby, more ways to study them (Lander et al., 2001).

Although Morgan earned his Nobel prize for establishing a cellular basis of heredity, his contributions did not stop there. He introduced *Drosophila melanogaster* as a valuable animal model for the study of genetics, a model that is still widely used today. In addition, Morgan's and his students' discovery of genetic linkage and recombination has had invaluable application in genetic mapping studies. Together with the discovery of polymorphic markers such as restriction fragment length polymorphisms (RFLPs), microsatellites and single nucleotide polymorphisms, linkage analysis has enabled positional cloning and genetic mapping which historically, has been an important means of gene identification for several disorders (Altshuler et al., 2008, Pulst, 1999).

Furthermore, genetic mapping has been used in combination with *physical mapping* techniques to derive gene maps. Gene maps describe the spatial arrangement of genes on chromosomes. Physical mapping differs from genetic mapping. Whilst genetic mapping uses genetic techniques, such as examination of pedigrees and cross-breeding, to derive the positions of genes relative to one another, physical mapping employs molecular biology techniques, derived from recombinant DNA technology, such as restriction mapping, fluorescent *in-situ* hybridization (FISH) and sequence tagged site (STS) mapping to determine the precise physical location of a given gene on a chromosome. Refinement of some of the techniques used to derive gene maps culminated in the successful completion of the Human Genome Project in 2003 (Chial, 2008), with sequencing of the entire human genome (approximately 3 billion bases), identification of the physical location of each gene, and better characterization of sequence variants (Lander et al., 2001, Kidd et al., 2008).

With the advent of the Human Genome Project, next-generation sequencing technologies (Slatko et al., 2018) have rapidly evolved. These have provided ever cheaper and faster ways of sequencing the entire human genome, thereby accelerating the discovery of genes associated with human diseases.

1.4.2 The importance of genetic discovery – clinical implications for epilepsy

Due to the impressive advancement of molecular genetic technologies in recent years, new epilepsy genes are now being discovered at a precipitous pace. Already, more than

500 genes have been associated with epilepsy and more than 80 genes have been associated with developmental and epileptic encephalopathies (McTague et al., 2016, Devinsky et al., 2018, Heyne et al., 2018)

The discovery of each new causative gene for an epilepsy syndrome is important for 3 reasons: (i) it enables clinicians to provide some answers for patients and their families, (ii) it provides insights into physiological gene function and pathophysiological mechanisms underpinning epilepsy; and (iii) it paves the way for development of targeted treatment strategies.

1.4.2.1 Providing answers for patients and their families

Arrival at a genetic diagnosis carries several benefits for affected patients and their families. It is well recognized that most patients with rare chronic neurological disorders experience an arduous diagnostic odyssey filled with uncertainty, and repeated hospital admissions for often painful, invasive investigations, such as lumbar punctures and skin or muscle biopsies. A genetic diagnosis brings an end to this difficult journey, providing much needed "closure" for many families. It can offer reassurance to parents who may be pondering if, somehow, their child's difficulties were due to some personal oversight; and allows both the family and the clinician more time and energy during clinic consultations to consider (disease-specific) management options (Ngoh and Parker, 2017).

Furthermore, sometimes establishing a genetic diagnosis can allow future prognostication regarding typical disease trajectory for a specific genetic condition. Co-morbidities may thus be anticipated, and the family and clinician can plan the surveillance and management of these in advance. In some cases, a definitive genetic diagnosis can even improve access to services such as physiotherapy, occupational therapy, and speech and language therapy.

Lastly, making a genetic diagnosis facilitates genetic counselling, providing families with more accurate information regarding recurrence risk in future pregnancies, as well as future options of pre-implantation diagnosis and prenatal testing

1.4.2.2 Insights into mechanisms involved in neurodevelopment and epilepsy pathophysiology

Whilst our knowledge is far from complete, genetic discovery in the developmental and epileptic encephalopathies has given us precious insight into mechanisms underlying both epilepsy and neurodevelopment.

As already discussed in (**Section 1.3.1**), the regulation of excitatory/inhibitory neural activity and processes crucial to normal cognitive development share a common dependence on the integrity of brain structure and function. Structural causes of epilepsy are classed separately from the developmental and epileptic encephalopathies and are generally associated with a distinct group of genes. Causative genes for developmental and epileptic encephalopathies tend to have roles in processes associated with efficient synaptic transmission, which also involves a balance between LTP and LTD. These genes may be grouped by function into genes encoding: (i) ion channels, (ii) cellular transporters, (iii) synaptic proteins involved in vesicle formation, endocytosis or exocytosis, (iv) proteins for intracellular signalling, and (v) genes with key roles in the regulation of gene/ protein expression and distribution (**Table 1-1**).

Table 1-1: Causative Genes for Developmental and Epileptic Encephalopathies: Illustrated Examples Classified by Neuronal Function

Gene (MIM number)	Protein Function	Phenotype (MIM number)	Mode of inheritance	Key References
Proteins involved in ion channel function				
CACNA1A (601011)	Alpha 1A subunit of a calcium voltage- gated channel (VGCC)	EIEE 42 (617106)	AD	(Epi4K Consortium, 2016, Epi4K Consortium et al., 2013)
FHF1 (FGF12) (601513)	Fibroblast growth factor homologous factor. Interacts with the cytoplasmic tail of voltage gated sodium channels and positively regulates their activity, enhancing neuronal excitability	EIEE 47 (617166)	AD	(Siekierska et al., 2016, Al-Mehmadi et al., 2016, Guella et al., 2016, Villeneuve et al., 2017, Takeguchi et al., 2018)
FRRS1L (604574)	Ferric Chelate Reductase 1 Like. A component of the outer core of the AMPA receptor protein. Modulates glutamate signalling	EIEE 37 (616981)	AR	(Madeo et al., 2016, Shaheen et al., 2016)
GABRA1 (137160)	Alpha-1 subunit of gamma-aminobutyric acid (GABA) Type A receptor.	EIEE 19 (615744)	AD	(Carvill et al., 2014, Farnaes et al., 2017, Johannesen et al., 2016)
GABRB1 (137190)	Beta-1 subunit of GABA Type A receptor	EIEE 45 (617153)	AD	(Epi4K Consortium et al., 2013, Lien et al., 2016)
GABRB3 (137192)	Beta 3 subunit of GABA Type A receptor	EIEE 43 (617113)	AD	(Epi4K Consortium et al., 2013, Le et al., 2017, Moller et al., 2017, Papandreou et al., 2016) Epi4K 2016
GABRG2 (137164)	Gamma 2 subunit of GABA Type A receptor	GEFS (611277) EIEE 6/DS (607208)	AD	(Carvill et al., 2013a, Zou et al., 2017, Reinthaler et al., 2015)
GRIN1 (138249)	N1 subunit of the NMDA receptor	Non-specific NDD with hyperkinesia and sz (614254 and 617820)	AD /AR	(Hamdan et al., 2011, Ohba et al., 2015, Lemke et al., 2016, Chen et al., 2017a, Zehavi et al., 2017)
GRIN2A (138253)	N2A subunit of the NMDA receptor	EASD (245570)	AD	(Carvill et al., 2013b, Chen et al., 2017b, DeVries and Patel, 2013, Lemke et al., 2013, Lesca et al., 2013, Marwick et al., 2015, Pal et al., 2017, von Stulpnagel et al., 2017, Yang et al., 2017)

Gene (MIM number)	Protein Function	Phenotype (MIM number)	Mode of inheritance	Key References
GRIN2B (138252)	N2B subunit of the NMDA receptor	EIEE 27 (616139)	AD	(Lemke et al., 2014, Platzer et al., 2017)
HCN1 (602780)	Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1. Contributes to native autonomous currents in neurons and in the heart	EIEE 24 (615871)	AD	(Nava et al., 2014, Poduri, 2014, Steel et al., 2017)
KCNA2 (176262)	Encodes Potassium Voltage-Gated Channel Subfamily A Member 2. Has a role in repolarization after an action potential	EIEE 32 (616366)	AD	(Pena and Coimbra, 2015, Syrbe et al., 2015, Masnada et al., 2017, Steel et al., 2017, Hundallah et al., 2016, Corbett et al., 2016)
KCNQ2 (602235)	Potassium Voltage-Gated Channel Subfamily Q Member 2. Plays a role in determining sub-threshold excitability of neurons	EIEE 7 (613720)	AD	(Dedek et al., 2003, Borgatti et al., 2004, Weckhuysen et al., 2012, Saitsu et al., 2012, Bassi et al., 2005, Kato et al., 2013)
SCN1A (182389)	Sodium Voltage-Gated Channel Alpha Subunit 1. Essential for action potential generation	EIEE 6/DS (607208)	AD	(Fujiwara et al., 2003, Harkin et al., 2007, Carranza Rojo et al., 2011, Brunklaus et al., 2014)
SCN2A (182390)	Sodium Voltage-Gated Channel Alpha Subunit 2 Essential for action potential generation	EIEE 11 (613721)	AD	(Kamiya et al., 2004, Ogiwara et al., 2009, Howell et al., 2015, Sanders et al., 2018)
SCN8A (600702)	Sodium Voltage-Gated Channel Alpha Subunit 2 Essential for action potential generation	EIEE 13 (614558)	AD	(Veeramah et al., 2012, Carvill et al., 2013a, Ohba et al., 2014, Butler et al., 2017)
Transporter proteins				
SLC1A2 (600300)	Glutamate transporter	EIEE 41 (617105)	AD	(Epi4K Consortium, 2016, Guella et al., 2017)
SLC1A4 (600229)	Amino acid transporter, which transports L-serine, L-alanine, L-cysteine, and L-threonine. I	IS, non-specific DEE	AR	(Conroy et al., 2016)
SLC25A22 (609302)	Mitochondrial glutamate transporter	EIEE 3 (609304)	AR	(Molinari et al., 2005, Molinari et al., 2009, Poduri et al., 2013)
SLC6A1 (137165)	GABA transporter which removes GABA from synaptic cleft	EMAS (616421)	AD	(Carvill et al., 2015, Johannesen et al., 2018)

Gene (MIM number)	Protein Function	Phenotype (MIM number)	Mode of inheritance	Key References
Genes with roles in synaptic vesicle formation (Endocytosis/Exocytosis)				
AP3B2 (602166)	Involved in the formation of clathrin coated synaptic vesicles	EIEE 48 (617276)	AR	(Assoum et al., 2016)
DNM1 (602377)	Dynamin-1. Involved in clathrin-mediated endocytosis and other vesicular trafficking processes	EIEE 31 (616346)	AD	(EuroEPINOMICS-RES Consortium et al., 2014, The Deciphering Developmental Disorders Study, 2015, von Spiczak et al., 2017)
STXPB1 (602926)	Regulation of synaptic vesicle docking and fusion	EIEE 4 (612164)	AD	(Saitsu et al., 2008, Carvill et al., 2014)
SYNJ1 (604297)	A polyphosphoinositide phosphatase with a role in clalthrin mediated endocytosis	EIEE 53 (617389)	AR	(Hardies et al., 2016)
Genes involved in intracellular signalling pathways				
CDKL5 (300203)	A protein kinase that regulates neuronal morphology through intracellular signalling pathways	EIEE 2 (300672)	XLD	(Kalscheuer et al., 2003, Weaving et al., 2004, Tao et al., 2004, Bahi-Buisson et al., 2008, Fehr et al., 2013, Kilstrup-Nielsen et al., 2012)
DOCK7 (615730)	A guanine nucleotide exchange factor involved in the regulation of cortical neurogenesis	EIEE 23 (615859)	AR	(Perrault et al., 2014)
GNAO1 (139311)	Alpha subunit of guanine nucleotide-binding proteins (G proteins), involved in transmembrane cell signalling.	EIEE 17 (615473)	AD	(Nakamura et al., 2013, Saitsu et al., 2016, Danti et al., 2017, Schorling et al., 2017)
IQSEC2 (300522)	A guanine nucleotide exchange factor for the ARF family of small guanosine triphosphate (GTP) binding proteins. Has a role in cytoskeletal organization at the post synaptic density at excitatory synapses	Non-specific DEE IS, LGS	XLD	(Zerem et al., 2016, Zipper et al., 2017, Epi4K Consortium, 2016, Epi4K Consortium et al., 2013)
PLCB1 (607120)	Phospholipase C- Beta-1. Catalyzes the production of second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3)	EIEE 12 (613722)	AR	(Kurian et al., 2010, Poduri et al., 2012, Ngoh et al., 2014, Schoonjans et al., 2016)
SIK1 (605705)	A member of the AMP kinase subfamily. Plays a role in signalling pathways involved in gene expression	EIEE 30 (616341)	AD	(Hansen et al., 2015)

Gene (MIM number)	Protein Function	Phenotype (MIM number)	Mode of inheritance	Key References
SYNGAP1 (603384)	A Ras GTPase activating protein that has a role in LTP	Non-specific DEE	AD	(Mignot et al., 2016, von Stulpnagel et al., 2015)
TBC1D24 (613577)	GTPase-activating proteins, which regulates intracellular vesicular transport, actin modelling and neurite development	EIEE 16 (615338)	AR	(Duru et al., 2010, Milh et al., 2013, Balestrini et al., 2016, Ngoh et al., 2017)
WWOX (605131)	Plays a role in intracellular signalling and apoptosis	EIEE 28 (616211)	AR	(Abdel-Salam et al., 2014, Tarta-Arsene et al., 2017)
Genes with roles in the regulation of gene/ protein expression and distribution (transcription/translation/DNA repair)				
ARX (300382)	Transcription factor which plays essential roles in neuronal migration and maintenance	EIEE 1 (308350)	XLR	(Mirzaa et al., 2013, Kato et al., 2004, Tapie et al., 2017)
AARS (601065)	Amino-acyl tRNA synthetase	EIEE 29 (616339) Non-specific DEE	AR	(Simons et al., 2015)
*ALG13 (*300776)	Catalyzes asparagine (N)-linked glycosylation in endoplasmic reticulum, a modification that regulates protein folding and stability	EIEE 36 (300884) WS, LGS	XLD	(Epi4K Consortium et al., 2013, de Ligt et al., 2012, Michaud et al., 2014, Hino-Fukuyo et al., 2015)
BRAT1 (614506)	Involved in DNA repair pathways	Severe EIEE (614498)	AR	(Straussberg et al., 2015, Puffenberger et al., 2012, Saunders et al., 2012, Saitsu et al., 2014b)
CHD2 (602119)	Chromodomain Helicase DNA Binding Protein 2 Modifies chromatin structure thereby regulating gene expression	Childhood onset DEE (615369)	AD	(Rauch et al., 2012, Carvill et al., 2013a, Suls et al., 2013, Lund et al., 2014)
EEF1A2 (602959)	Eukaryotic translation elongation factor 1, alpha 2. Involved in delivery of aminoacyl tRNAs to ribosomes.	EIEE 33 (616409)	AD	(de Ligt et al., 2012, Veeramah et al., 2013, Lam et al., 2016)
FOXG1 (164874)	Forkhead Box G1. Functions as a transcription repressor	LGS, non-specific DEE	AD	(Lindy et al., 2018, Seltzer et al., 2014, Ma et al., 2016)
MEF2C (600662)	MADS Box Transcription Enhancer Factor 2, Polypeptide C. Plays an important role in neural crest and craniofacial development	Non-specific EIEE WS	AD	(Vrecar et al., 2017, Paciorkowski et al., 2013, Bienvenu et al., 2013)

Gene (MIM number)	Protein Function	Phenotype (MIM number)	Mode of inheritance	Key References
PNKP (605610)	Involved in DNA repair	LGS, non-specific DEE	AD	(Shen et al., 2010, Poulton et al., 2013, Nakashima et al., 2014)
PURA (600473)	Purine Rich Element Binding Protein A. Has a role in the control of DNA replication and transcription	LGS, non-specific DEE	AD	(Lee et al., 2018, Reijnders et al., 2018, Hunt et al., 2014)
QARS (603727)	Glutaminyl-tRNA Synthetase. Important role in protein translation	EIMFS, EIEE, non-specific DEE	AR	(Kodera et al., 2015, Zhang et al., 2014)
SMC1A (300040)	Cohesin multiprotein complex is required for sister chromatid cohesion	IS, focal infantile epilepsy EIMFS	X-linked dominant (truncating)	(Jansen et al., 2016, Symonds et al., 2017, Huisman et al., 2017, Gorman et al., 2017)
UBA5 (610552)	A ubiquitin-like post-translational modifier protein	EIEE 44 (617132)	AR	(Arnadottir et al., 2017, Colin et al., 2016)
Genes with roles in synaptic stability				
PCDH19 (300460)	Protocadherin 19. Ca^{2+} dependent cell adhesion molecules with a role in synaptic stabilization and neuronal network connections	EIEE 9 (300088)	X-linked (a cellular-interference model may explain why only heterozygous females and mosaic males are affected)	(Smith et al., 2018, Ryan et al., 1997, Scheffer et al., 2008, Depienne et al., 2009, Jamal et al., 2010, Pederick et al., 2018)
PIGA (311770)	Has a role in the synthesis of glycosylphosphatidylinositol (GPI), a glycolipid that attaches dozens of different proteins to the cell surface	WS, Non-specific DEE, EME	XLR	(Kato et al., 2014, Kim et al., 2016, Trump et al., 2016)
SPTAN1 (182810)	Filamentous cytoskeletal proteins that function as essential scaffold proteins	EIEE 5 (613477)	AD	(Tohyama et al., 2008, Nonoda et al., 2013, Syrbe et al., 2017)

AD: autosomal dominant, AR: autosomal recessive, DEE: developmental and epileptic encephalopathy, DS: Dravet syndrome; EASD: epilepsy aphasia spectrum disorders; EIEE: early infantile epileptic encephalopathy, EIMFS: Epilepsy of infancy with migrating focal seizures; EMAS: Epilepsy with myoclonic atonic seizures; EME: early myoclonic epilepsy, GEFS: Generalized epilepsy with febrile seizures ; IS: infantile spasms, LGS: Lennox Gastaut syndrome, NDD: neurodevelopmental delay; Sz: seizures; WS: West Syndrome; XLD: X-linked dominant, XLR: X-linked recessive

1.4.2.3 Development of targeted treatments: a "precision medicine" approach

The discovery of a new candidate gene for a given epilepsy syndrome usually prompts laboratory functional investigations to further characterize the pathogenic effects of identified gene variants. Understanding how these variants lead to disease enables the development of targeted therapies, which may be specifically tailored to each individual gene mutation – a so-called "precision medicine" approach.

Although such personalised medicine strategies are not available for all human diseases, gene-specific therapies are now beginning to emerge for the early onset epilepsies. For instance, quinidine, an anti-arrhythmic agent and potassium-channel antagonist, has been shown to reverse the gain-of-function effect of *KCNT1* mutations *in vitro.* Quinidine has been used to treat some cases of epilepsy related to *KCNT1* mutations, and reports have yielded mixed results (Milligan et al., 2014, Mikati et al., 2015, Fukuoka et al., 2016, Bearden et al., 2014, Mullen et al., 2018, McTague et al., 2018). In some patients, this therapy achieves seizure reduction, but in the majority it proves ineffective. One study investigating the effects of quinidine treatment in 43 *KCNT1*-postive patients reported a response rate (>50% seizure reduction) in 20% of patients (Fitzgerald et al., 2019). The sub-optimal efficacy of this therapy may be due to the fact that quinidine has low potency in inhibiting the sodium activated potassium sub-unit, $K_{Na}1.1$, *KCNT1* encodes (Cole et al., 2020). Additionally, it is not selective in inhibiting just $K_{Na}1.1$ in the central nervous system, and also exerts effects on cardiac channels. As such, the risk of affecting cardiac function limits dosing levels of quinidine (Mullen et al., 2018).

As another example, in tuberous sclerosis complex (TS), *TSC1* or *TSC2* mutations cause neuropsychiatric manifestations, including seizures, through suppressing the inhibition of the mechanistic target of rapamycin (mTOR) pathway leading to this pathway's over-activation (Inoki et al., 2002). mTOR inhibitors, everolimus and sirolimus have thus been studied as anti-epileptic drugs for TS patients of all ages with favourable results (Krueger et al., 2013, Krueger et al., 2016, Overwater et al., 2016, Samueli et al., 2016, French et al., 2016, Franz et al., 2018, Saffari et al., 2019, Stockinger et al., 2021). In particular,

the EXIST-3 trial, a phase 3 double-blind, randomized, placebo-controlled trial demonstrated that everolimus used as adjunctive therapy in TS drug-refractory epilepsy resulted in sustained time-and-exposure-dependent reduction in seizures in patients aged 2 years and older (Franz et al., 2018, French et al., 2016). As a result of this trial, in December 2016, everolimus was approved by the European Medicines Agency for "*adjunctive treatment of patients aged 2 years and older whose refractory focal- onset seizures, with or without evolution to bilateral tonic-clonic seizures, are associated with tuberous sclerosis complex*" (Curatolo et al., 2018).

Finally, sodium channel blockers have been shown to aggravate epilepsies associated with loss-of-function *SCN1A* mutations (de Lange et al., 2018). In contrast, sodium channel blockers have been found to be effective in epilepsies associated with gain-of-function *SCN2A* and *SCN8A* mutations (Wolff et al., 2017, Moller and Johannesen, 2016). These drugs have also been found to be effective in *KCNQ2*-related epilepsy (Pisano et al., 2015), possibly attributed to the co-localization and interaction of voltage gated potassium and sodium channels.

1.5 Landau Kleffner Syndrome

Landau Kleffner Syndrome (LKS) has been considered a prototypical epileptic encephalopathy. Affected children have an often profound regression of speech and language abilities in association with characteristic epileptiform discharges on EEG. Recently, some cases of LKS have been reported to have genetic aetiology, making it therefore a developmental and epileptic encephalopathy.

It has been more than 60 years since William Landau and Frank Kleffner first published a case series of six children, describing what has subsequently been named Landau Kleffner syndrome (Landau and Kleffner, 1957). William Landau and Frank Kleffner observed that whilst most children educated in the Institute for the Deaf in St Louis, Missouri, had congenital hearing loss, there were also a few children who had an acquired deficit, with disease onset later in childhood, associated with a seizure disorder. These children had all developed speech and language normally in their early years, then subsequently lost this ability.

Landau and Kleffner suggested in their landmark paper that "*persistent convulsive discharge in brain tissue largely concerned with linguistic communication results in the functional ablation of these areas for normal linguistic behaviour*".

Today, more than 60 years after LKS was first described, our understanding of this clinically recognizable syndrome remains relatively limited. This may in part be due to the fact that LKS is extremely rare. A recent epidemiological study in Japan estimated the incidence of this disorder to be approximately 1 in 978,000 (Kaga et al., 2014).

LKS is rare, and as such, some may argue that it contributes relatively little to the morbidity of the general population. However, enhancing our understanding of such rare disorders is likely to have wider relevance. Indeed, as one of the prototypical developmental and epileptic encephalopathies, what we learn from mechanisms underlying LKS, can contribute to our overall understanding of how factors such as genetics and epileptic activity, influence neurodevelopment and cognitive outcome.

1.5.1 Clinical Presentation of LKS

The clinical features of the first six cases described by William Landau and Frank Kleffner are summarized in **Table 1-2** (Landau and Kleffner, 1957).

Consistent features within the cohort include: (i) normal early development; (ii) speech and language regression (age range 4 - 9 years, mean 6.5 years); (iii) a seizure disorder with abnormal electroencephalogram (EEG), (iv) maintenance of non-verbal cognitive abilities; (v) some recovery of speech and language, either spontaneously or with anti-convulsant therapy; and (vi) relatively easily controlled seizures.

Table 1-2: Summary of first 6 cases originally reported by William Landau and Frank Kleffner in 1957

Case	Early dev	FH	PMH	Preceding factors	Age at Sz	Age at SLR	Sz	Other dev	EEG	Treatment	Course of illness/outcome
1	N	+ve*	NIL	Skin infection, and fall preceded SLR	4y	5y	NT-GTCS, FM	BD	GSW, > over TR	PHT, PHB, PD	Recurrent episodes. 1st: resolved with PHT/PHB. 2nd: 5m, later, dense aphasia with FS, helped by PD/SLT
2	N	+ve*	FS at 8y	2 episodes of minor HI preceded SLR regression	11y	9y	NT- FM	NIL	R- SWD > over central regions	PHT, PHB	Recurrent episodes. 1st after HI: abN EEG but resolved spontaneously. 2nd: after sz onset, gradual improvement with AEDs/SLT
3	N	NIL	NIL	NIL	3.5y	5y	NT-GTCS, FM, MC, AS	NIL	Frequent GSW	PHT, PHB, PD	SLR occurred while Sz were controlled on AEDs. Aphasia improved on AED escalation/SLT
4	N	NIL	NIL	Fall without LOC before SLR	4.5y	4y	FM	BD, MD	GSW > on R, > over TR	PD, PMT, PHT PHB	Recovered some speech on AED, but relapsed. Some Sz while on AEDs. Gradual improvement with SLT, but continued to have SLI
5	N	NIL	NIL	Mumps infection	9y	7y	GTCS	NIL	GSW, TRS	MPB	SLR at 7y. Single Sz at 9y then nil after MPB started. No change for 1 year then gradual improvement with SLT over 1-2y.
6	N	NR	NIL	Headaches and visual disturbance	9y	9y	TS, FM	BD, B-IQ	TRS, GSW	NR	Onset of Sz followed by BD and expressive aphasia. Continued to have receptive understanding. Some spontaneous improvement but speech returned to N with AEDs

abN: abnormal; AED: Anti-epileptic drug; AS: absence seizures; BD: behavioural difficulties; B-IQ: borderline IQ; FH: family history, FM: focal motor; GTCS: generalised tonic-clonic seizures; GSW: generalised spike-waves; HI: head injury; LOC: loss of consciousness; MC: myoclonic; MD: movement disorder; MPB: mephobarbitone; N: normal; NT: nocturnal; NR: not reported; PD: paradione; PHT: phenytoin; PHB: phenobarbitone; PMH: past medical history; R: right; SLI: speech and language impairment; SLR: speech and language regression; SLT: speech and language therapy; SWD: spike-wave discharges; Sz: seizures; TS: tonic seizures; TR: temporal regions; TRS: temporal spikes . *Case 1 and 2 are brothers, their father had seizures at age 47y; their mother and younger brother had abnormal EEGs, but no clinical seizures.

To date, approximately 350 cases of Landau Kleffner syndrome have been described in the literature (Tuft et al., 2015). The definition of the disorder has varied slightly between different publications, but most experts agree that the hallmark feature defining this disorder is an acquired aphasia associated with a seizure disorder (in turn, defined as clinical seizures and/or characteristic epileptiform activity on EEG). As noted in Landau and Kleffner's original report, there may not be a clear temporal correlation between the presentation of aphasia and onset of seizures or discharges on EEG (Van Bogaert, 2013, Landau and Kleffner, 1957).

Table 1-3 summarizes the clinical features described in previous LKS cohort studies. As more cases are described, it becomes increasingly evident that, within the framework of an acquired aphasia associated with a seizure disorder, the syndrome is clinically heterogeneous.

From what is currently known, the age of onset varies from 18 months to 14 years, with peak onset between 5 and 7 years. Earlier studies with small numbers favoured a male predisposition, however, larger cohort studies have shown a more even gender distribution (**Table 1-3**).

From the 1970s, it was clear that not all children with LKS had clinically manifesting seizures. Currently, it is estimated that 70-80% of individuals with LKS have clinical seizures (Fandino et al., 2011, Pearl et al., 2001). Focal seizures are most commonly reported, these are often nocturnal, with or without secondary generalization. Other reported seizure types include atypical absences, generalized tonic-clonic and myoclonic seizures. In teenage years, the EEG commonly normalizes and clinical seizures cease (**Section 1.5.2**).

Although earlier studies mainly reported LKS children with normal early development, many later reports include children with some early developmental delay, including speech and language delay. Indeed, in some cohort studies, such patients comprise up to 75% of the cohort (**Table 1-3**).

Regression of speech and language skills seem to occur gradually for some children and more acutely for others. For most children, receptive language skills are more severely

affected than expressive skills, but in a smaller subset, expressive difficulties are more prominent (Stefanatos, 2011). Both receptive and expressive language skills can be affected to different extents. Some children experience a dense auditory agnosia- not only do they not understand speech, they also fail to make sense of every day environmental sounds and music (Baynes et al., 1998); others do not understand speech but preserve the ability to interpret environmental sounds and/or appreciate music. Some are able to comprehend simple verbal instructions. Expressive difficulties range from relatively subtle word finding difficulties, to stuttering, unintelligible vocalization and grunting. Some patients speak gibberish with no discernible words but maintain a normal voice quality and inflection, whilst others may speak recognizable words but with aberrations in pitch, volume and/or inflection.

In Landau and Kleffner's original report, all patients were reported to have preserved non-verbal skills. Two patients in the original report had behavioural difficulties, notably hyperactivity, tantrums, and poor tolerance to change in routine. In subsequent reports, a significant proportion of patients had associated non-verbal cognitive deficits **(Table 1-3)**. On average, approximately 60-70% of patients appear to have co-morbid behavioural difficulties including aggression, irritability, attention deficit hyperactivity disorder, and autistic traits **(Table 1-3).**

1.5.2 Electroencephalogram (EEG) findings in LKS

Characteristic EEG features are a cardinal feature of LKS. Over the last six decades, the interictal EEG findings reported in children with LKS have not deviated significantly from those described in the original paper by Landau and Kleffner (**Table 1-2**) (Landau and Kleffner, 1957).

The most frequently reported EEG abnormalities are epileptiform discharges with a central or temporal lobe predominance around the Sylvian fissure occurring unilaterally or bilaterally (Tuft et al., 2015). However, a large variety of EEG abnormalities have been reported including generalised or multifocal epileptiform discharges (Stefanatos, 2011).

By the late 1970s it was recognized that the interictal EEG abnormalities found in LKS activate significantly in non-rapid eye movement (REM) sleep (Gascon et al., 1973, Kellermann, 1978, Rodriguez and Niedermeyer, 1982). Indeed, it was recognized that

some children only demonstrate sleep-activated anomalies (Genton et al., 1992). During non-REM sleep, the paroxysmal activity typically becomes more generalized and symmetrical. For extended periods of time, during non-REM sleep, spike-wave discharges can become continuous. If continuous spike and wave activity occurs over more than the arbitrarily assigned proportion of 85% of slow-wave sleep (also known as a spike-wave index of more than 85%), the EEG meets criteria for electrical status epilepticus in slow wave sleep (ESES), first described in 1971 in a syndrome known as epilepsy with continuous spike-waves in slow wave sleep (ECSWS) (Patry et al., 1971).

LKS differs clinically from ECSWS as children with ECSWS have more widespread developmental regression including cognitive, motor and behavioural disturbances **(Section 1.5.3).** The EEG in LKS may also differ slightly from that of ECSWS as ESES activity tends to be maximal over posterior temporal and parietal electrodes in LKS, and maximal over frontal-central regions in ECSWS.

An example of ESES is presented in **Figure 1-4**.

Figure 1-4: Sleep EEG demonstrating electrical status epilepticus in slow wave sleep (ESES)

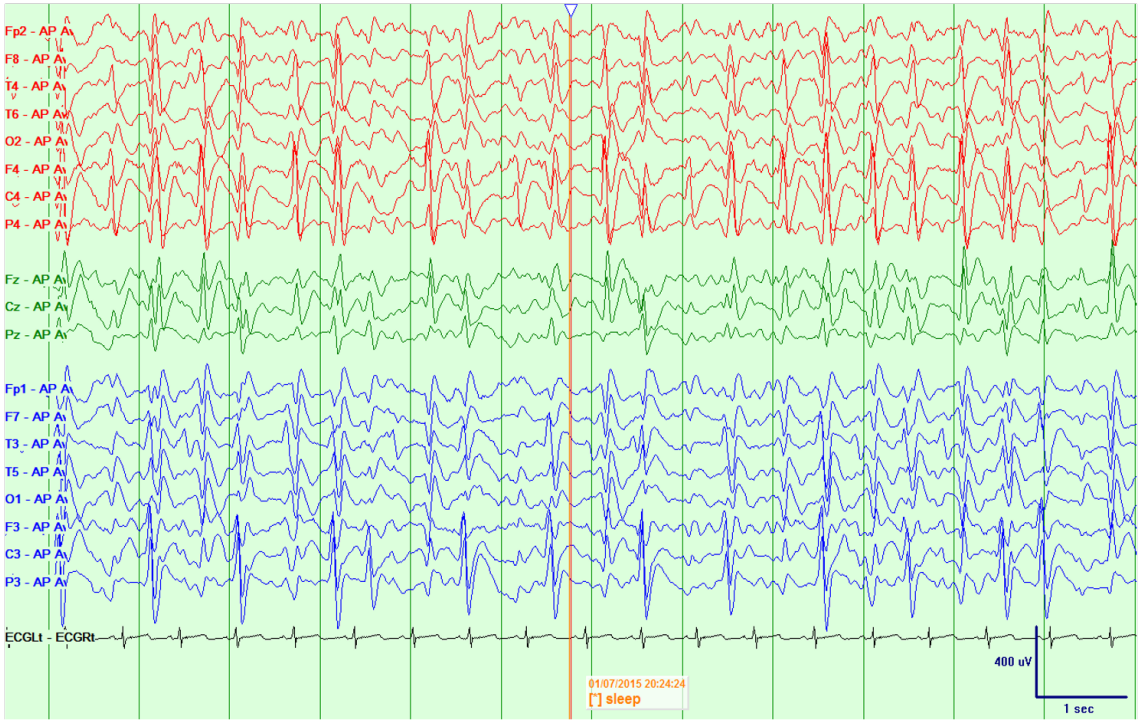


Table 1-3 Summary of clinical features reported in LKS

Study	(Mantovani and Landau, 1980)	(Deonna et al., 1989)	(Soprano et al., 1994)	(Rossi et al., 1999)	(Duran et al., 2009)	(Robinson et al., 2001)	(Cockerell et al., 2011)	(Caraballo et al., 2014)
N.	9	7	12	11	7	17	19	29
Male	33.3%	85.7%	75%	63.6%	100%	35%	NR	68.9%
Mean length of FU (range)	21y9m (10y to 28y)	18y6m (13y to 27y6m)	8y11m (2y6m to 15y4m)	9y8m (2y3m to 16y2m)	13y3m (5y to 19y)	5y 7m (1y to 15y)	10y9m (1m to 25y)	12y (3-21 y)
Positive FH	22.2%	NR	NR	45.5%	NR	11.7%	NR	Not reported
Pre-existing SD	0%	14.2%	75%	27.3%	NR	23.5%	0%	65.5%
Mean age at SLR (range)	5y9m (3y to 9y)	5y (3y to 8y)	4y10m (4y to 5y6m)	3y5m (1y6m to 5y8m)	4y6m (3y to 9y)	4y8m (2y1m to 7y)	3y7m (1y5m to 6y)	5y (2y to 9y)
Clinical Sz	66.6%	85.7%	58%	81.8%	85.7%	88.2%	47%	79.4%
ESES on EEG (SA)	NR	NR	NR	100%	28.6% (71.4%)	NR (100%)	NR (89.5%)	51.7% (41.3%)
< average NVS	0%	28.6%	50%	81.8%	NR	11.8%	15.7%	48.3%
BD at presentation	44.4% (HA/Agg)	71.4% (Agg)	75% (HA)	72.7% (> 1: Agg, ASD, ADHD, HA)	NR/42.8% at last FU (HA, ASD)	52.9% (Agg/ ASD, HA)	89.5% or > (Agg, ADHD, HA)	65.5% (ADHD, Agg,)
Language OC at last FU	NL: 44.4% MI: 11.1% MDI: 44.4%	NL: 28.6% MI/MDI: 14.3% SI/NS: 42.9%	NL: 25% MI/MDI:57.9% SI: 16.6%	NL: 18.2% MDI: 45.5% SI: 36.3%	NL: 14.3% MI- SI: 85.8% (42.9% PR)	NL: 17.6% MDI: 23.5% SI/NS: 23.5%*	NL: 21% MDI: 58% SI/NS: 21%	NL: 27.5% MI-SI: 73.5%
Sz at last FU	0%	14.3%	Not reported	0%	28.6%	Not reported	0%	10.3%
Outcome at >18y	N: 7 NL/I: 57.1% MI/I 14.3% MDI/I: 28.6%	N.: 7 I: 42.9% D: 57.1%	N: 3 NL: 66.6% MDI: 33.3% I: NR	N.: 1 SI: 100% I: NR	N.: 4 NL/I: 25% MI – SI/D: 75% (25% PR)	N.: 0	N.: 4 NL/I: 75% MI/I: 25%	No.: 13 NL/I: 46.2% MI-MDI/I: 30.8% SI/D: 23.1%

ADHD: attention deficit hyperactivity disorder; Agg: aggression; ASD: autistic traits; BD: behavioural disorder; D: dependent; ESES: electrical status epilepticus in sleep (85% of spike wave index); FH: family history of seizures or speech and language impairment; FU: follow-up; m: months; HA: hyperactivity; I: independent; MI: mild speech impairment; MDI: moderate speech impairment; N.: number of patients; NL: normal language; NR: not reported; NS: no speech; NVS: non-verbal skills; OC: outcome; PR: partial remission; SD: speech delay; SLR: speech and language regression; SA: sleep activation (<85% spike wave index); SI: severe speech impairment; Sz: seizures; y: years *remainder: not reported

1.5.3 LKS and the Broader Epilepsy Aphasia Spectrum

Similar EEG findings and overlapping clinical features have led to the proposal that LKS belongs within a spectrum of disorders –the spectrum of idiopathic focal epilepsy with rolandic spikes, or the epilepsy aphasia spectrum. On the mild end of this continuum lies childhood epilepsy with centrotemporal spikes (CECTS). In this syndrome, children present with nocturnal focal motor seizures and epileptiform discharges localized over the rolandic region; however, they do not have ESES, and they do not normally show significant developmental regression. LKS lies on the severe end of this spectrum, along with Epilepsy with Continuous Spike-Waves in Slow Wave sleep (ECSWS). As its name suggests, the latter syndrome features continuous spike-waves in sleep, otherwise known as ESES. In contrast to LKS, children with ECSWS often have refractory epilepsy with more global developmental regression including cognitive, motor and behavioural impairment.

There is controversy over whether <85% of epileptiform activity in non-REM sleep or unilateral epileptiform activity is compatible with a diagnosis of ESES, and indeed, debate whether the presence of ESES is actually necessary for a diagnosis of LKS. As such, intermediate disorders termed “intermediate epilepsy aphasia disorder (IEAD)” and “atypical benign partial epilepsy (ABPE)” have now emerged. These disorders are characterized by some features of language and/or cognitive regression, associated with rolandic spikes on EEG that activate in sleep, but do not meet the specific ≥ 85% spike wave-index criterion for ESES (Carvill et al., 2013b).

1.5.4 Aetiology

The aetiology and pathogenesis of LKS remain poorly understood. One of the earliest theories postulated that encephalitis was the underlying cause, though examination of post-resection epilepsy surgery specimens did not support this theory (Cole et al., 1988).

Historically, other proposed aetiologies for LKS include neurocysticercosis (Otero et al., 1989), toxoplasmosis (Michaoowicz et al., 1988), temporal ganglioglioma (Mikati et al., 2009), haemophilus influenza meningitis (Pearl et al., 2001), subacute sclerosing panencephalitis (Pearl et al., 2001), cerebral arteriopathy (Pascual-Castroviejo et al.,

1992) and inflammatory demyelination (Perniola et al., 1993). However, many authors preferred to define LKS as an "idiopathic" (cryptogenic) focal epilepsy without structural abnormality on cranial imaging (Hirsch et al., 2006). In recent years, the two most popular theories for the underlying aetiology of LKS have been that it is either an autoimmune or genetic disorder.

Some studies have reported the presence of autoantibodies directed against various cerebral antigens in LKS (Connolly et al., 1999, Connolly et al., 2006, Boscolo et al., 2005). The possibility of LKS being an autoimmune disorder is also supported by the observation that many patients respond favourably to immunomodulation with corticosteroids and intravenous immunoglobulins. However, others have claimed that intense epileptic activity in LKS may itself bring about a secondary inflammatory process, which can be modulated by steroid therapy, and therefore steroid efficacy may not be synonymous with an autoimmune aetiology. It is also important to note that a significant proportion of individuals with LKS do not respond to steroid therapy.

A genetic aetiology for LKS is supported by its age-limited predilection suggesting a developmental disorder. In addition, although Landau and Kleffner's original series only had 1 pair of siblings with a positive family history, subsequent cohort studies have reported a family history of either febrile seizures, epilepsy or speech and language disorders in up to 45% of patients (Rossi et al., 1999) **(Table 1-3).** Furthermore, an excess of rare copy number variants are reported in patients with LKS and ECSWS (Lesca et al., 2012). In 2013, an underlying genetic aetiology was confirmed by reports of mutations in *GRIN2A* [encoding the GluN2A subunit of the N-methyl-D-Aspartate (NMDA) receptor] in 8- 20% of individuals with LKS and other related epilepsy aphasia syndromes (Lesca et al., 2013, Lemke et al., 2013, Carvill et al., 2013b).

1.5.5 Treatment of LKS

Limited understanding of LKS pathogenesis translates to a lack of targeted treatment options. As such, treatment has largely been empirical.

As patients with LKS have prominent language, cognitive and behavioural impairments that may be more disabling for these children and their families than just clinical seizures, the goal of treatment is not just to reduce seizure frequency but also to

produce developmental gain. Alongside medical or surgical treatment, it is vital not to overlook the importance of regular speech and language, neurocognitive and behavioural assessments with multi-disciplinary therapy as required.

Over the last 6 decades, pharmacological treatments that have been offered to patients include benzodiazepines, other anti-epileptic drugs (e.g. valproate, ethosuximide, sulthiame, levetiracetam), hormonal therapy (corticosteroids, adrenocorticotrophic hormone) and intravenous immunoglobulins. Non-pharmacological therapies that have been explored include the ketogenic diet, vagal nerve stimulation and epilepsy surgery with multiple sub-pial transections (MST).

When interpreting all studies gathering evidence for therapies in LKS, it is important to keep in mind the natural relapsing-remitting nature of LKS in its active phase. Some results may relate to coincident natural remission, rather than the efficacy of the treatment in question.

Table 1-4 presents a summary of the literature available for some pharmacological treatments currently used for LKS. Most of these represent open label studies or anecdotal reports (Class IV evidence). Randomised controlled trials remain rare. A recent Cochrane study aiming to review randomised controlled trials for pharmacological treatments for LKS and ECSWS did not find any relevant, completed studies (Moresco et al., 2020). Due to the lack of secure evidence for any one pharmacological treatment, no standardised approach for the treatment of LKS exists today. Some authors reviewing LKS, and other epileptic encephalopathies with ESES, have suggested that the use of benzodiazepines or corticosteroids may be preferable options for the improvement of both seizures and speech/neuropsychological difficulties (Hughes, 2011, Veggiotti et al., 2012, Baumer et al., 2021, van den Munckhof et al., 2015). Currently, a European multi-centre randomised controlled clinical trial is underway to compare the efficacy of corticosteroids versus clobazam in epileptic encephalopathy with ESES (van den Munckhof et al., 2020).

The ketogenic diet often offers positive results for drug refractory epilepsy (McDonald and Cervenka, 2020). However, not much data is currently available on its efficacy in LKS. In 1999, Bergqvist et al reported that use of the classical ketogenic diet resulted in improvement in seizure control, language function and behaviour for 3 patients with LKS

who did not respond to anti-epileptic drugs and adrenocorticotrophic hormone (Bergqvist et al., 1999). Vagal nerve stimulation has also been undertaken in the management of LKS. Similarly, there is limited data on its efficacy in LKS. In 2003, Park et al reported that of 6 children with LKS treated with vagal nerve stimulation, 3 achieved at least a 50% reduction in seizure frequency at 6 months, with corresponding improvement in quality of life .

Surgical treatment for LKS was first proposed by Frank Morrell in 1995 on the premise that LKS was likely to be due to an epileptogenic lesion over cortical regions governing speech and language (Morrell et al., 1995). He proposed a technique known as multiple subpial transection which involved transecting intracortical transverse fibres whilst sparing vertically directed connections for neuronal pathways. The rationale behind this approach was that selective disruption of transverse fibres would stop the spread of epileptiform activity but sparing of vertically aligned pathways would preserve the physiological function of eloquent cortex (Morrell et al., 1995). Whilst earlier studies suggested some promise for this approach (Morrell et al., 1995, Grote et al., 1999, Cross and Neville, 2009), a more recent study reported insufficient evidence that MST offers significant benefit over and above recovery observed in comparable patients who do not undergo surgery (Downes et al., 2015).

Table 1-4: Reports on efficacy of pharmacological treatments used for LKS

Treatment	Evidence
Benzodiazepines	**(De Negri et al., 1995)**: Rectal diazepam (1mg/kg) was administered to 43 children with various epilepsy syndromes during EEG monitoring. 25 responded, among them, 1 child with LKS, and 15 with ECSWS. The responders were given cycles of 2-4 weeks of oral diazepam (0.5-0.75mg/kg/d) as further treatment, and the child with LKS were among the 64% of children who showed a positive response with at least 50% decrease of the SWI and neuropsychological improvement. **(Marescaux et al., 1990):** Clobazam was administered to 3 patients with LKS at doses of 1-1.6mg.kg/d. Patients 1 and 3 showed improvement in EEG findings, whilst patients 1 and 2 showed marked improvement in language skills. These effects were transient in all patients disappearing after 3-8 weeks of treatment. Clonazepam (0.1mg/kg/d) was administered to 1 patient, resulting in transient improvement in EEG and language skills for 3 months before relapse.
Other anti-epileptic drugs	**(Marescaux et al., 1990) :** Phenobarbitone, phenytoin and carbamazepine aggravate both clinical symptoms an EEGs in LKS. Valproate (30-50mg/kg/d) was shown to be effective at improving seizures, EEG and neuropsychological impairment in 1/5 patients and to improve seizures and EEG but not language or behaviour in 1 patient. Ethosuximide (20mg/kg/d) in combination with valproate improved seizures and EEG but not language or behaviour in 2/2 patients. **(Holmes and Riviello, 2001**): In a retrospective study, valproic acid (started at 10-20mg/kg/d) was found to be effective in improving language skills in 40% of 57 children with LKS, particularly in those with early onset aphasia. The presence of seizures and EEG findings were not significantly different between those with improved language skills and those without. **(Wirrell et al., 2006):** In 1 child with LKS, sulthiame at 10mg/kg/d improved language and resolved EEG abnormalities (**Wakai et al., 1997)**: In 1 child with LKS who did not respond well to valproate, ethosuximide, carbamazepine, thyrotropic releasing hormone and high dose immunoglobulin, the administration of sulthiame resulted in immediate improvement in EEG, seizure frequency, language skills and behaviour **(Kossoff et al., 2003):** In 1 child with LKS, starting Levetiracetam (60mg/kg/d) and discontinuation of valproate and carbamazepine resulted in improvement in both seizure frequency and language **(Larsson et al., 2012)** In a double-blind placebo controlled randomised cross-over trial involving 18 children with nocturnal epileptiform activity, including some fitting criteria for LKS, Levetiracetam was found to be effective in reducing EEG spike-wave index.

Treatment	Evidence
Corticosteroid therapy	**(McKinney and McGreal, 1974) :** 9 children with LKS were reported. 3 children were given ACTH (+/- prednisone or IVIG) and made rapid full recovery. 1 child recovered on AED therapy alone. **(Lerman et al., 1991):** Reported 4 children with LKS, 1 was given ACTH, 2 were given prednisone and 1 given dexamethasone- all had prompt recovery of both speech difficulties and EEG **(Marescaux et al., 1990):** 3 children with LKS were given hydrocortisone and/or prednisone. All recovered normal EEGs and had improved language skills. 1 developed Cushing's syndrome and osteoporosis. **(Santos et al., 2002):** 3 patients with LKS were administered prednisone in addition to various AEDs. All 3 had improvement in seizure control and language skills. 1 patient was only given Carbamazepine, she also became seizure free and had some improvement in language skills. (**Sinclair and Snyder, 2005)**: 8 patients with LKS were given prednisone. All but 1 had significant improvement in language skills, cognition and behaviour sustained over follow- up periods of 1- 10 years. (**Tsuru et al., 2000)**: 2 patients with LKS who had seizure control on anti-epileptic drugs were given iv methylprednisolone for 3 consecutive days followed by oral prednisolone for 1 month before weaning off. Both patients achieved improvement of speech and language skills.
Intravenous immunoglobulin (IVIG)	**(Fayad et al., 1997) :** 1 child with LKS who did not respond to AEDs and prednisone, was reported to have sustained response after treatment with 3 courses of IVIG (**Lagae et al., 1998)**: 1 child who did not respond to AEDS and relapsed after 2 courses prednisone achieved longer remission of symptoms with a 5 day course of IVIG. (**Mikati and Saab, 2000)**: 1 child with LKS with no prior therapy with other agents had normalised EEG and language skills after treatment with 4 days of IVIG therapy. He relapsed 2 months later but responded to a further course of IVIG (**Mikati et al., 2002**): Assessed the efficacy of 5 LKS patients given IVIG 2g/kg over 4-5 days. 2 patients responded well with complete resolution of aphasia.

ACTH: adrenocorticotrophic hormone; AED: anti-epileptic drug; ECSWS: Epilepsy with continuous spike waves in sleep; EEG: electroencephalogram; IVIG: intravenous immunoglobulin; LKS: Landau Kleffner Syndrome; SWI: spike-wave index

.

1.5.6 Long Term Outcome of LKS

The long-term disease trajectory and clinical outcome varies significantly amongst individuals with LKS **(Table 1-3).** Overall, approximately 1/3 of individuals regain normal speech and language skills. Another 1/3 respond to treatment sub-optimally and gradually recover some speech and language skills. The remaining do not respond to treatment and do not recover useful language into adulthood. A proportion of the latter, however, still manage to live independent lives within the deaf community (discussed further in Chapter 3).

1.6 Summary

This introductory chapter has reviewed research undertaken over more than 2 centuries, providing insight into the multifaceted mechanisms governing neurocognition and epilepsy pathogenesis, concepts that are clearly exemplified by the electroclinical disorder, LKS. Furthermore, it has provided an opportunity to consider what it means for families when a child is diagnosed with a developmental and epileptic encephalopathy for which, due to our current limited understanding, only empirical therapy is available.

So much remains to be learnt before we can start to make a real difference for these children and their families. However, as we build on the accretion of previous knowledge, our learning accelerates. Through the clinical and molecular genetic investigation of a large cohort of patients with LKS, the following chapters aim to further contribute to our current understanding of this syndrome, and the mechanisms underlying developmental and epileptic encephalopathies.

2 Chapter 2: General Materials and Methods

2.1 Overall study design

This PhD study comprised 2 main parts:-

1. A clinical endophenotyping cohort study to (i) better characterize clinical features of Landau Kleffner Syndrome (LKS), (ii) determine what factors may influence outcome, and (iii) define phenotypic features associated with specific genetic sub-groups.
2. Molecular genetics analysis to evaluate the role genetics plays in the aetiology and pathogenesis of LKS.

2.1.1 Literature review

Electronic literature searches were carried out on the PubMed database to gain better understanding of what is currently known about Landau Kleffner syndrome. No date or language restrictions were set. Key search terms included the medical subject headings (MeSH): "Landau Kleffner Syndrome" and "epilepsy-aphasia spectrum disorder". Special note was taken of previous clinical cohort studies, for comparison with this current LKS cohort.

2.1.2 Patient recruitment

Patients referred for LKS were recruited from a database of children treated at the GOSH Developmental Epilepsy Clinic (DEC) from January 1990 to June 2018.

In October 2016, an LKS family day was held by GOSH DEC. All past and present patients on the database, with available contact details, were invited. More than 100 people attended. During this day, talks were given about LKS from both the professional multi-disciplinary team and from patients/families recounting their own experience. This LKS research study was introduced, and some families were recruited during this event.

2.1.3 Consent and Ethics Approval

All patients were either given information about the research study at their clinic appointment, or contacted by telephone/ postal mail.

Written informed consent was obtained from all participants, and the study was approved by the Local Research Ethics Committee (REC reference 13/LO/0168, IRAS project ID 95005). All clinical research adhered to principles outlined by the Declaration of Helsinki.

2.1.4 Acquisition of Blood samples from Study Participants

Blood samples were obtained from all affected individuals, as well as both parents and all consenting siblings.

2.1.5 Inclusion criteria

For the LKS clinical cohort study, only patients meeting strict inclusion criteria for LKS were included. These inclusion criteria were: (i) a speech and language led regression; and (ii) evidence of a consistent seizure disorder around the time of regression (i.e. clinical seizures and/or centrotemporal spikes with sleep activation or ESES).

During clinical endophenotyping, patients who were found to have long-standing speech and language impairment without a clear history of regression and patients who had clinical features more in keeping with ECSWS, with a more global or non-verbal regression, were excluded from the clinical cohort study. As clinical endophenotyping took place alongside blood collection for deoxyribonucleic acid (DNA) extraction, some of these individuals would already have had blood samples taken. These individuals remained included for molecular genetics analysis.

2.2 Clinical Cohort Study Data Collection

2.2.1 Patient Assessment

From the start of my PhD, wherever possible, I reviewed newly referred patients in the Developmental Epilepsy Clinic (DEC). I took a detailed medical and family history, performed a systemic and neurological examination, and observed assessments carried out by speech and language therapists and clinical neuropsychologists. Where face-to-

face review was not possible (e.g. for patients not under active follow-up), I carried out a detailed retrospective case note review.

EEG data from all patients was extracted from formal EEG reports.

All study data was pseudo-anonymized. Each patient was assigned a unique identifier code, and clinical data was entered into an excel database for further analysis. I utilized the Statistical Product and Service Solutions (SPSS) statistical programme, to derive statistical values including frequency, mean values, standard deviation and range.

2.2.2 Assessment of Disease Outcome

I obtained results for language outcomes and non-verbal skills by extracting data from formal speech and language and neuropsychological assessments undertaken by specialists in the DEC clinic.

Inevitably, the tests used to assess these parameters differed from patient to patient as the most appropriate test had to be chosen based on the child's age and estimated abilities. In addition, testing material has been modified and updated in the time course over which this cohort was collected (1990 – 2018).

Tests used to assess speech and language include British Picture Vocabulary Scale (BPVS), BPVS-II, Test for Reception Of Grammar, Renfrew Action Picture Test, Clinical Evaluation of Language Fundamentals (CELF)-Revised, CELF-Preschool, Pre-school CELF-2, CELF-3, and CELF-4. Tests used to assess non-verbal skills in this study include Ravens coloured progressive matrices, Hickey Nebraska test of learning aptitude, McCarthy's scales of children's abilities, Wechsler abbreviated scale of intelligence (WASI), Wechsler Intelligence Scale for Children (WISC) III, WISC-IV, Wechsler Preschool and Primary Scale of Intelligence (WPPSI)-Revised and WPPSI-IV. Most children received a combination of more than one method of testing during the course of their illness.

Language outcomes were classified as: average, mild impairment, moderate impairment, severe impairment and no functional language as defined in **Table 2-1.**

As results obtained from different assessments were not always truly comparable, where possible, percentile measures were obtained for each respective test, based on

the average score expected for the child's age, as an approximate means for comparison. Where only age-equivalent results were available, age equivalence of half the chronological age or less was taken to define severe language impairment as suggested by previous literature (Robinson et al., 2001). Age equivalence of more than half but less than 75% of the chronological age was arbitrarily defined as moderate language impairment. Where more than one assessment was used during the same clinic attendance and a range of scores was obtained, e.g. receptive language 25th centile, and expressive language 10th centile, the worse/worst score (10th centile) was recorded for the purpose of this analysis.

Non-verbal skills were classified as: (i) average or above average and (ii) below average.

Table 2-1: Classification of Language Outcomes

Classification	Percentile	Standard deviation (SD)	Age equivalence
Average/Normal language	> 25th centile	Within 1 SD	Age equivalent
Mild impairment	12.5th centile to 25th centile	Between -1 to - 1.5 SD	>75% of chronological age
Moderate impairment	1st centile to 12.5th centile	Between - 1.5 to 2 SD	50% to 75% of chronological age
Severe impairment	< 1st centile	< 2 SD or unable to use age appropriate scale	< 50% chronological age
No functional speech	Unable to verbalize or difficult to decipher single words		

A proportion of the cohort were diagnosed and treated as children, but are now adults (>18 years of age). These patients were contacted via telephone or email to ascertain their level of independence, level of speech and language difficulties (formal speech and language assessments were not repeated) and seizure burden, if any. Their outcomes as adults were classified as defined in **Table 2-2.**

Table 2-2: Classification of outcomes as adults

Category	Definition
Independent, mild or no language difficulty	In employment or in college, normal speech and language skills or only minor difficulties not significantly affecting lifestyle
Independent with severe speech and language impairment	In employment or in college but with severe speech and language difficulties (e.g. using sign language) and/or frequent seizures
Dependent	Not in employment or in college. Dependent on parental care or in residential care.

2.3 Molecular Genetics Analysis

2.3.1 Materials

2.3.1.1 Chemical Reagents

Agarose	Bioline
Ampicillin sodium salt	Sigma Aldrich
Anti-GRIN2A antibody produced in rabbit	Sigma Aldrich
Anti-GRIN2A extracellular antibody produced in rabbit	Alomone labs
2x Biomix$_{TM}$ Red	Bioline
Clarity Western ECL Substrate	Bio-Rad
D-2-amino-5-phosphonopentanoic acid (D-AP5)	Sigma Aldrich
Distilled water	Gibco
Dithiothreitol (DTT)	ThermoFisher
DMEM	Gibco
100bp DNA Ladder with 6x Loading Dye	Promega
EDTA 0.02% Solution Cell Culture Tested	Sigma Aldrich
Ethanol 100%/70%	Haymankimia
Fetal Bovine Serum (FBS)	Gibco
GAPDH HRP Conjugate Antibody	Cell Signaling Technology
GC rich solution (FastStart Taq DNA Polymerase Kit)	Roche
Goat anti-rabbit HRP conjugated secondary antibody	Abcam
HEK293 cells (ATCC® CRL-1573)	ATCC

Laemmli Sample Buffer 4X	Bio-Rad
LB (Lysogeny broth) Powder	Sigma Aldrich
LB Agar (Powder)	Sigma Aldrich
Lipofectamine 2000 Transfection reagent	ThermoFisher
MESA Blue qPCR MasterMix	Eurogentec
MicroCLEAN ®	Web Scientific
Paraformaldehyde, 16% w/v aq. soln., methanol free	ThermoFisher
Pencillin-Streptomycin (10,000units/ml)	Life Technologies
Phosphate Buffered Saline (PBS)	Gibco
Pierce protease and phosphatase inhibitor	ThermoFisher
Precision Plus Protein Dual Colour Standards	Bio-Rad
Primers	Sigma Aldrich
Radioimmunoprecipitation assay (RIPA) buffer	Sigma Aldrich
SYBR Safe DNA Gel Stain	ThermoFisher
Sodium Acetate 3M	Ambion
1 x Tris-Borate EDTA Electrophoresis Buffer	Geneflow
Tris-EDTA Buffer 1 x pH 8.0	Applichem
Tris-Glycine/SDS buffer 10x diluted to 1x	Bio-Rad
Wheat Germ Agglutinin, CF488A conjugate	Biotium

2.3.1.2 Kits

Big Dye Terminator v1.1 Cycle Sequencing Kit	ThermoFisher
Pierce BCA Protein Assay kit	ThermoFisher
QuantiTect Reverse Transcription Kit	Qiagen
Qubit dsDNA BR Assay Kit	Invitrogen
Quikchange Lightning Site Directed Mutagenesis Kit	Agilent Technologies
QIAprep Spin MiniPrep Kit	Qiagen
HiSpeed Plasmid Maxi Kit	Qiagen
RNeasy Mini Kit	Qiagen
SALSA MLPA P410 GRIN2A GRIN2B probemix	MRC Holland
SALSA MLPA EK1 reagent kit -100rxn- FAM	MRC Holland

2.3.2 DNA Sequencing

Direct Sanger Sequencing were carried out to (i) specifically screen for *GRIN2A* mutations and establish the frequency of *GRIN2A* mutations in the cohort of recruited patients; (ii) confirm candidate gene variants identified on whole exome sequencing/whole genome sequencing analysis (WES/WGS); and (iii) establish familial segregation for identified *GRIN2A* variants and candidate gene variants identified on WES/WGS.

2.3.2.1 DNA Extraction and concentration measurement

Lymphocytic DNA was kindly extracted by the Great Ormond Street Hospital North East Thames Regional Genetics Service Laboratory using standard methods.

Aliquots of DNA were measured using the Qubit fluorometric quantitation method to determine DNA concentration. The Qubit dsDNA BR Assay Kit (Invitrogen) was used according to the manufacturer's protocol. In general, DNA stock concentrations were approximately 150-250ng/µl. These were diluted to provide working concentrations of 20-40ng/µl for polymerase chain reaction (PCR) and Sanger sequencing purposes.

2.3.2.2 Primer Design

The genomic DNA template for each gene was obtained from Ensembl genome browser (http://www.ensembl.org/index.html). Based on all Ensembl coding transcript variants, I designed primer pairs for exon-specific PCR amplification of genomic exons and flanking intron boundaries using Primer3 software (http://bioinfo.ut.ee/primer3/).

Primer sequences for *GRIN2A* sequencing are listed in Chapter 4 **(Table 4-3).**

2.3.2.3 Polymerase Chain Reaction (PCR) Amplification

PCR amplification was performed in 20µl reactions as follows:-

2x BioMix™ Red (Bioline Ltd.)	10.0µl
Distilled water dH_2O	7.2µl
Forward primer	0.4µl of 10µM concentration
Reverse primer	0.4µl of 10µM concentration
Genomic DNA (20ng/µl)	2.0µl

For Guanine-Cytosine (GC)-rich DNA fragments, PCR amplification was modified to 20µl reactions as follows:-

2x BioMix™ Red (Bioline Ltd.)	10.0µl
Distilled water dH_2O	3.2µl
GC rich solution	4.0µl
Forward primer	0.4µl of 10µM concentration
Reverse primer	0.4µl of 10µM concentration
Genomic DNA (20ng/µl)	2.0µl

All PCR reactions were prepared in thin-walled non-skirted 96-well plates (ThermoFisher). For each primer pair, a DNA sample from a normal unaffected individual, and a DNA-free sample (where 2µl dH_2O was added instead of DNA) were included as a positive and negative control respectively.

PCR amplification was performed using an Applied Biosystems Thermocycler. The following conditions were used: an initial denaturation of 95°C for 5 minutes, followed by 40 cycles of 45 seconds denaturation at 95°C, 45 seconds annealing at 58°C to 60°C (annealing temperature was optimized specifically for each primer pair) and 1 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes.

2.3.2.4 Agarose Gel Electrophoresis

PCR products were separated on 1.5% horizontal agarose gels to check the PCR reactions worked without contamination. Agarose (1.5g) was melted in 1x TBE buffer (100ml) in a domestic 800W microwave and cooled; then 5µl of SYBR Safe Gel Stain was added before casting the gel in a gel-casting tray. Plastic combs were inserted to create 4 rows of 12 wells each in the gel, and the gel was left to solidify for 20 minutes under an extraction hood. After the gel has solidified, the gel-tray was placed in an electrophoresis tank and 5ul of PCR product was loaded directly to each appropriate well. 5µl of 100bp DNA sizing ladder with 6x loading dye was added to each row to establish correct PCR product size. Electrophoresis at 120V was carried out for 30 minutes to achieve DNA separation. Bands were then imaged using ChemiDoc™ MP Imaging System (Biorad), and analysed using ImageLab software.

2.3.2.5 PCR Product Clean Up and Cycle Sequencing

PCR products were cleaned up using MicroCLEAN (Web Scientific). 3.5 µl of MicroCLEAN was added to 3.5µl of PCR product in a thin walled non-skirted 96 well plate and centrifuged at 4,000rpm at room temperature for 40 minutes. The supernatant was discarded by spinning the plate upside down at 500rpm for 30 seconds at room temperature. The pellet was then reconstituted with 4.5µl of dH_2O.

The purified PCR product was subsequently sequenced in both forward and reverse directions in an Applied Biosystems Thermocycler, using the appropriate primers.

10µl sequencing reactions were set up as follows:-

Purified PCR product	4.5µl
Forward or Reverse primer	1µl (10µM concentration)
5x BigDye Sequencing buffer	2µl
Distilled water dH_2O	2µl
Big Dye v1.1	0.5µl

Cycle sequencing conditions were: 96°C for 3 min, followed by 35 cycles of 96°C for 30 seconds, 50°C for 15 seconds, and 60 °C for 4 minutes.

2.3.2.6 Precipitation of Sequenced DNA

The sequencing reactions were then cleaned up to remove any incorporated dye terminators. 2µl of sodium acetate and 50µl of 100% ethanol were added to 10µl of sequencing reaction. The plate was vortexed before incubation for 20 minutes at room temperature. The sequencing plate was then centrifuged at 3000rpm for 40 minutes at 4^{o}C, and the supernatant was discarded by spinning the plate upside down at 300rpm for 1 minute. Subsequently, 50µl of 70% ethanol was added and the plate was centrifuged again at 3000rpm for a further 10 minutes. The supernatant was then discarded by spinning the plate upside down at 300rpm for 1 minute. Following this, the plate was air-dried to remove any excess alcohol, and the DNA pellet was re-suspended in 10µl of 1:10 TE buffer solution.

2.3.2.7 Sequencing reaction and analysis

Sequencing reactions were run on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems Inc.), by the Great Ormond Street Hospital North East Thames Regional Genetics Service Laboratory. Sequencing data was then analyzed using one of the following softwares:

Chromas	http://www.technelysium.com.au/chromas.html
Sequencher	https://www.genecodes.com
Mutation Surveyor	https://softgenetics.com/mutationSurveyor.php

2.3.3 Multiplex Ligation Probe Amplification (MLPA)

MLPA was carried out to look for *GRIN2A* copy number variants that may have potentially been missed on Sanger sequencing. This was performed in collaboration with the Great Ormond Street Hospital (GOSH) for Children/ North East Thames Regional Genetics Service using an MLPA kit (SALSA MLPA P410 GRIN2A GRIN2B probemix and SALSA MLPA EK1 reagent kit, MRC Holland) according to manufacturer's instructions. The P410-A1 probemix contains 17 probes for the *GRIN2A* gene covering all exons with the exception of exon 2. I observed these experiments and participated in the analysis of the results.

2.3.3.1 DNA Denaturation, Hybridization and Ligation

5 samples of control DNA, kindly provided by GOSH/North East Thames Regional Genetics service and 1 blank control (1:10 TE with no DNA), were included for each MLPA run of 50 samples.

250ng of sample DNA diluted in 1:10 TE (50ng/ul) was denatured at 98°C for 20 minutes, then the thermocycler was paused at 25 °C while 3µl of probe mix from the kit was added. The samples were denatured again at 98°C for 1 minute, then hybridized at 60°C for 16 hours. 32µl of ligation master-mix from the kit, containing 1µl of Ligase-65 enzyme, was added to each sample and annealed probes were ligated at 54 °C for 20 minutes, before ligase was inactivated by incubating at 95°C for 5 minutes.

2.3.3.2 PCR amplification

10µl of polymerase master mix (comprising 7.5µl dH_2O, 2µl SALSA PCR primer mix from the kit, and 0.5µl SALSA polymerase from the kit), was added to each reaction at room temperature and mixed.

PCR amplification was carried out under the following conditions: 95°C for 5 minutes, 34 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 1 minute; then 72 °C for 20 minutes.

2.3.3.3 Fragment separation and results analysis

The reaction products were separated by capillary electrophoresis on ABI PRISM® 3790. The results were analysed using GeneMarker V2.4.0 software (SoftGenetics, MRC Holland).

2.3.4 Whole exome sequencing/Whole genome sequencing

When this project started March 2015, the first batch of patients who tested negative for *GRIN2A* mutations were sent for familial triome whole exome sequencing (WES) in order to look for other candidate genes. By 2016, as whole genome sequencing (WGS)

technologies became increasingly available, we subsequently sent patients who screened negative for *GRIN2A* mutations for familial triome WGS. Some patient DNA samples were acquired towards the end of the project. Due to budget constraints, DNA from these families was sent for proband only WES.

I undertook this part of the project in collaboration with Hywel Williams at GOSGene, UCL-GOS Institute of Child Health.

For both WES and WGS, 2µg of genomic DNA (50ng/µl) sample was sent to Beijing Genomics Institute (BGI)- Hong Kong. Library construction was performed there. Samples from each triome were sequenced per lane to an overall coverage of 100x using the Illumina HiSeq 2500 sequencing platform.

Following sequencing at BGI-Hong Kong, raw data was returned to GOSgene in .fastq file format (2x per sample). First pass quality control analysis was undertaken by Hywel William using GOSgene's established bioinformatic pipeline (Mestek-Boukhibar et al., 2018), which includes mapping of reads to the reference genome, calling and recalibration of variant calls and the production of a list of filtered high quality variants. I then uploaded the variant call files (.vcf) into the Qiagen Ingenuity Variant analysis software platform and further filtered the genomic data using a range of specific parameters, before analysis and interpretation (**Chapters 5 and 6**).

2.3.5 Screening for copy number variants

All families who underwent whole genome sequencing were screened for structural variants using LUMPY (Layer et al., 2014). This was performed by bioinformaticians at GOSgene, UCL Institute of Child Health. Briefly, LUMPY is a flexible probabilistic structural variant discovery framework used for the detection of genomic deletions, duplications, insertions, inversions, and translocations. It integrates multiple structural variant detection signals generated from whole genome sequencing data, including altered sequence coverage (read-depth), read-pairs and split reads. With high coverage WGS data (10x to 50x) LUMPY's sensitivity for all structural variant types has been reported to be as high as 88.8% to 99.6%. Its false detection rate was reported to rise with higher coverage, but overall, remained relatively low, ranging from 0% to 7.1% (Layer et al., 2014).

For families who had whole exome sequencing (instead of whole genome sequencing) copy number variant analysis with LUMPY was not possible. One of these individuals had copy number variant screening via single nucleotide polymorphism array analysis performed by his referring hospital's diagnostic cytogenetics laboratory.

2.3.6 In Situ Site Directed Mutagenesis

In order to further understand the effect of *GRIN2A* variants on protein function, I undertook in situ site-directed mutagenesis to generate plasmids harbouring the 2 *GRIN2A* missense variants, detected in the patient cohort through *GRIN2A* screening.

Professor Robert Harvey (University of the Sunshine Coast, Australia) kindly provided the prk5 plasmid vectors harbouring wild-type *GRIN2A* DNA templates, for these experiments. The prk5 plasmid vector contains a cytomegalovirus promoter, a multiple cloning region with several restriction sites, a bacteriophage f1 origin of replication for production of single-stranded plasmid DNA, and an ampicillin resistant (AmpR) gene (**Figure 2-1**).

Mutant plasmids were validated, then transfected into Human Embryonic Kidney (HEK-293) cells to create an *in vitro* over- expression cellular model to study mutant N2A function (**Figure 2-2).**

Figure 2-1 prk5-*GRIN2A* plasmid map (https://www.snapgene.com/)

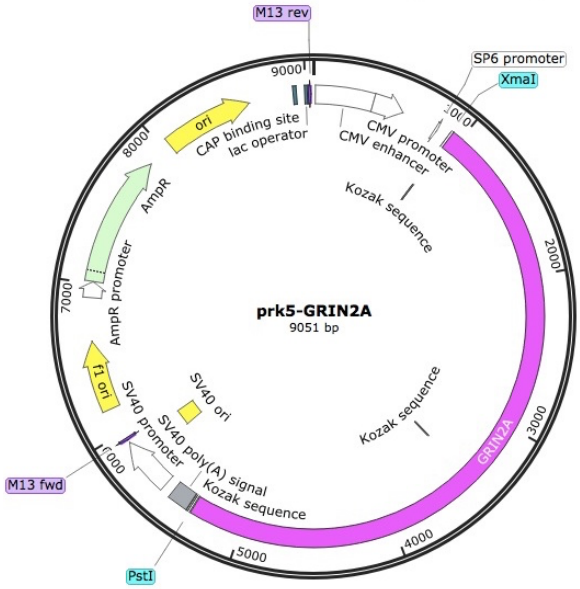
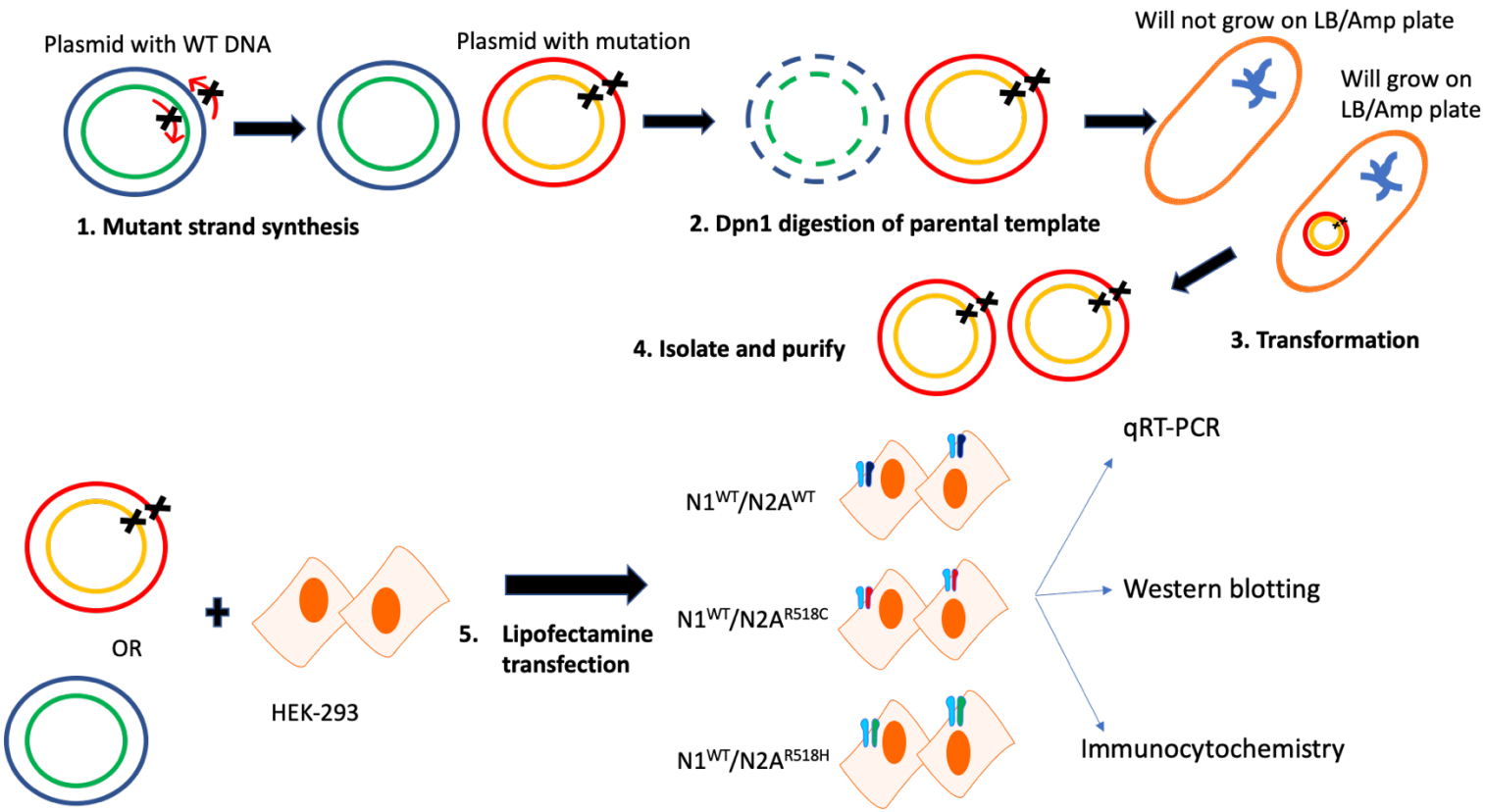


Figure 2-2 Schematic for site-directed mutagenesis and transfection of HEK-293 cells



1. Mutagenic primers containing desired mutation are annealed and extended with DNA polymerase, giving rise to plasmids harbouring the desired mutation. 2. The parental methylated DNA template is digested using *Dpn*1. 3. Mutagenized plasmids are transformed into E-coli/competent cells for amplification and nick repair. Only cells that take up the plasmid containing an Ampicillin resistant gene will colonize in LB/Ampicillin plates. 4. Colonies forming on LB/Ampicillin plates are picked and the mutagenized plasmid DNA is isolated and purified. 5. HEK-293 cells are transfected with either mutant (R518C/R518H) plasmids or WT plasmids to derive cells with WT or mutant N2A receptors for use in qRT-PCR, Western blotting and immunocytochemistry studies.

2.3.6.1 Preparation of material for bacterial culture

(i) LB (Lysogeny broth)

25g of LB powder (Sigma-Aldrich) was weighed and added to 1 litre of water. The solution was then autoclaved for sterilization.

(ii) Ampicillin solution

1 gram of ampicillin sodium salt (Sigma Aldrich) was added to 10mls of distilled water under a fume hood. The solution was then passed through a washed 0.22 µm filter for sterilization before being divided into 1ml aliquots (100mg/ml) and stored at -20°C.

(iii) LB Ampicillin Agar Plates

6.25g of LB powder and 3.75g of LB agar powder were added to 250ml of water and the solution was autoclaved for sterilization. Once cooled to approximately 40-50 °C, 250µl of ampicillin solution (100mg/ml) was added under a fume hood. The solution was then poured out into 10cm petri-dishes under a fume hood and left to solidify.

2.3.6.2 Mutagenic Primer Design

The DNA template of *GRIN2A* was taken from Ensembl genome browser (http://www.ensembl.org/index.html), NCBI Genome Reference Consortium (GRC)h38.p7; chromosome 16: 9,753,404 – 10,182,754; NM_000833.4. Mutagenic primers were designed using Stratagene's web based QuikChange Primer Design Program (http://www.stratagene.com/qcprimerdesign). Mutagenic primer sequences are presented in **Table 2-3**.

Table 2-3: *GRIN2A* Mutagenesis primers

Mutation	Primer Pair	Annealing temperature
p.R518C, c.1552C>T	Forward: 5'-gtccaccacttcagaacattcctcattgatggtga-3' Reverse: 5'-tcaccatcaatgaggaatgttctgaagtggtggac-3'	55°C
p.R518H, c.1553G>A;	Forward: 5'-caccatcaatgaggaacattctgaagtggtggact-3' Reverse: 5'-agtccaccacttcagaatgttcctcattgatggtg-3'	55°C

2.3.6.3 Mutant Strand Synthesis Reaction

Site directed mutagenesis was carried out using the Quikchange Lightning Site Directed Mutagenesis Kit. With complementary mutagenesis primers, this kit uses a high-fidelity non-strand displacing polymerase to amplify plasmid DNA in a thermocycler, creating a nicked circular DNA with the desired mutation.

PCR amplification was carried out in 50µl thin-walled tubes in an Applied Biosystems Thermocycler. 25µl reactions were set up as follows:-

10x reaction buffer from Quikchange Lightning kit	2.5µl
dsDNA template	50ng (0.5µl of 100ng/µl)
Forward primer	1.0µl of 10µM concentration
Reverse primer	1.0µl of 10µM concentration
dNTP mix from Quikchange Lightning kit	0.5µl
Quiksolution Reagent	0.75µl
Quikchange Lightning enzyme	1.0µl
Distilled water dH_2O	18.75µl

2.0µl of water was added in place of primers for negative controls.

The following thermocycling conditions were used:

Segment	**Cycles**	**Temperature**	**Time**
1	1	95°C	2 min
2	18	95°C	20 seconds
		55°C	10 seconds
		68°C	5 min
3	1	68°C	5 min

2.3.6.4 DpnI Digestion of Amplification Products

The restriction enzyme *Dpn*I was then used to remove the parental template DNA from the amplification products leaving only the mutated plasmids. As the parental template DNA was synthesized within *E.coli* bacteria, it is methylated and can be digested with

methylation sensitive *Dpn*I. However, as the mutated plasmids were generated *in vitro*, they are un-methylated and can circumvent digestion.

For this step, 1µl of *Dpn*I restriction enzyme (10U/µl) was added directly to a half-volume (12.5µl) of each amplification and control reaction. Each reaction mixture was gently and thoroughly mixed by pipetting up and down several times. The reaction mixtures were then spun down for 1 minute and incubated at 37^{o}C for 5 minutes to digest the parental template dsDNA.

2.3.6.5 Transformation of XL10- Gold Ultracompetent Cells

For amplification of the mutated plasmids, XL-10 Gold Ultracompetent cells provided within the Quikchange Lightning Site Directed Mutagenesis Kit were transformed with the *Dpn*I digested PCR products.

First, the XL-10 Gold Ultracompetent cells were gently thawed on ice. Following this, 2µl of β- ME mix was added to 45µl aliquots of ultracompetent cells in pre-chilled Eppendorf tubes. The tubes were swirled gently then incubated on ice for 2 minutes. 2µl of *Dpn*I treated DNA from each sample and control reaction were then added to separate aliquots of ultracompetent cells. The tubes were gently swirled and incubated on ice for a further 10 minutes. Each tube was then heat-pulsed at 42°C for 30 seconds, before incubation in ice for 2 minutes. 500µl of LB broth, pre-heated to 42°C, was then added to each tube before incubation at 37°C for 1 hour, shaking at 225-250rpm.

After incubation, 250µl of each transformation reaction was plated onto LB-ampicillin agar plates and incubated overnight at 37°C.

The following day, 2 to 3 discrete colonies from each sample plate were picked and added separately to 5ml of LB broth with 5µl of ampicillin (100mg/ml) in 15ml falcon tubes. These were left to culture for 6 to 8 hours at 37°C with shaking at 220 to 250rpm. Following this, these mini-cultures were used to inoculate 250ml of LB broth with 250µl ampicillin (100mg/ml) in 1-litre Erlenmeyer flasks and left to culture overnight at 37°C with shaking at 220 to 250rpm.

2.3.6.6 Plasmid DNA Isolation – MaxiPrep

After overnight incubation, plasmid DNA was extracted using the HiSpeed Plasmid Maxi-Kit (Qiagen).

The bacterial cells were harvested by centrifugation at 4000g for 30 minutes at 4°C. The supernatant was then discarded, and the cell pellets were frozen at -20°C for at least 3 to 4 hours, to aid cell lysis.

Following this, each bacterial pellet was re-suspended in 10ml Buffer P1 with pre-added RNAse A solution and LyseBlue. 10ml of Buffer P2 was added and the suspension was mixed thoroughly by vigorously inverting the sealed falcon tube 4 to 6 times, before incubation at room temperature (15–25°C) for 5 min. After incubation, 10ml of Buffer P3 was added and the cell-lysate was mixed by vigorous inversion once more before being poured into the barrel of a sealed QIAfilter cartridge and incubated for 10 minutes. During these 10 minutes, a HiSpeed Maxi Tip was equilibrated by applying 10ml Buffer QBT and allowing the column to empty with gravity. After 10 minutes of incubation, the cap was removed from the nozzle of the QIA filter cartridge and the cell lysate was poured into the equilibrated HiSpeed tip and filtered through this by gravity flow. The HiSpeed Maxi tip was then washed with 60ml of Buffer QC, before plasmid DNA was eluted with 15ml of Buffer QF. 10.5ml of isopropanol was added before incubation in room temperature for 5 minutes. After incubation, the eluate/isopropanol mixture was transferred to a syringe with a QIA precipitator attached and was filtered through the QIA precipitor using constant pressure. The trapped DNA was then washed, by passing 2ml of 70% ethanol through the QIA precipitor using constant pressure. The membrane of the QIA precipitor was dried, and finally, purified DNA was eluted into a 1.5ml collection eppendorf tube with 1 ml of Buffer TE.

This DNA was measured using NanoDrop, then sequenced using cDNA primers to confirm the presence of the full-length *GRIN2A* cDNA insert with the desired mutations. cDNA primer sequences are presented in **Table 2-4**.

Table 2-4 *GRIN2A* cDNA primers

Gene	cDNA Primer Pair	Annealing temperature
GRIN2A	Forward: 5'-caatgggaagcatggcaaga-3' Reverse: 5'-gctagccaggaatatgacagc-3'	60°C

2.3.7 HEK-293 Cell Culture and Transfection

To develop the over-expression cell-models for study of mutant *GRIN2A* expression and function, I co-transfected HEK-293 cells with a wild-type N1 construct, plus either a wild-type N2A construct, or a N2A construct containing one of 2 missense mutations found in the GOSH cohort (p.R518C and p.R518H). These gave me 3 populations of cells to use for reverse-transcription PCR, Western blotting and immunocytochemistry experiments (**Figure 2-2**).

2.3.7.1 Cell-culture

HEK-293 cells were maintained in 25cm^2 Corning tissue culture flasks (ThermoFIsher) containing 5ml of complete media- Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin (10,000units/ml). The cells were incubated in a 5% CO_2 incubator at 37°C and passaged twice a week.

2.3.7.2 Plating of cells

When the HEK293 cells were 80-90% confluent in the 25cm^2 flask, they were split into either Corning 6-well polystyrene plates (ThermoFisher) or 4-well Lab-tek chamber slides for transfection. Cells transfected in 6-well plates were used for Western blotting or for reverse transcription PCR (RT-PCR) experiments. Cells transfected in 4-well Lab-tek chamber slides were fixed for immunocytochemistry studies.

The cell-culture medium was aspirated from the 25cm^2 flask. 1ml of EDTA 0.02% Solution was added before the flask was returned to the incubator for 5 minutes of incubation at 37°C and 5% CO_2. During these 5 minutes, 2ml of complete media was added to each of 4 wells in a 6-well polystyrene cell culture plate, 0.5ml of complete media was added to each well of a 4-well Lab-tek chamber slide, and 5ml of complete

media was added to a fresh 25cm^2 flask. After 5 minutes incubation, the 25cm^2 flask was gently tapped at the sides to detach the cells, then the cells were re-suspended in the 1ml of EDTA 0.02% solution in the flask. 90µl of this 1ml of re-suspended cells was added to each of the 4 wells in the 6 well plate filled with complete media; 20µl was added to each well of the Lab-tek chamber slide, and a further 50 µl was added to the new 25cm^2 flask to maintain the cell-culture. The newly plated cells were then all returned to the incubator for incubation at 37^{o}C and 5% CO_2.

2.3.7.3 Transfection

When the cells in the 6-well plate and Lab-tek chamber slide were approximately 80% confluent (usually after overnight incubation), transfection was carried out using Lipofectamine 2000 transfection reagent.

All N1 and N2A plasmid DNA samples were diluted to a standard concentration of 100ng/ul.

The plasmid DNA part of a transfection cocktail was prepared as follows:-

6 well plate (per well):	8µg of N1 plasmid DNA made up to 100µl volume using serum-free DMEM, **and** 8µg of N2A WT **or** N2A mutant plasmid made up to 100µl volume using serum-free DMEM. Negative control: 200µl of serum free medium
Lab-tek slide (per well):	2µg of N1 plasmid DNA made up to 25µl volume using serum-free DMEM **and** 2µg of N2A WT **or** N2A mutant plasmid made up to 25µl volume using serum-free DMEM Negative control: 50µl of serum free medium

The lipofectamine part of the transfection cocktail was prepared as follows:-

6 well plate (per well):	10µl Lipofectamine 2000 reagent and 190µl of serum free DMEM
Lab-tek slide (per well):	2.5µl Lipofectamine 2000 reagent and 47.5µl of serum free DMEM

The plasmid DNA and lipofectamine parts of the transfection cocktail were then mixed together and incubated for 5 minutes at room temperature. After 5 minutes of incubation, 400µl of mixed transfection cocktail was added to each appropriate well in the 6-well plate, and 100µl of mixed transfection cocktail was added to each appropriate

well in the lab-tek chamber slide. Each 6-well plate/Lab-tek slide included one well of negative control cells – treated only with lipofectamine and serum free medium.

To protect the cells from NMDA-receptor mediated cell toxicity (Sibarov et al., 2017), 16µl of the NMDA receptor antagonist, D-2-amino-5-phosphonopentanoic acid (D-AP5) (200µmol/l) was added to each well of the 6 well plate, and 4µl of D-AP5 (200µmol/l) was added to each well of the Lab-tek chamber slide.

The cells were then returned to the incubator for incubation at 37^{o}C and 5% CO_2.

2.3.7.4 Harvesting transfected cells for Reverse Transcription-PCR (RT-PCR) and Western Blotting

24 hours after transfection, cell-culture medium was aspirated, and 1ml of EDTA 0.02% solution was added to each well in the 6-well plate. The plate was then returned to the incubator for 5 min incubation at 37^{o}C and 5% CO_2. Following this, the plate was gently tapped at the sides to detach the cells. The cells were then re-suspended in the EDTA 0.02% solution in the wells and collected into 1.5ml Eppendorf tubes.

The Eppendorf tubes were centrifuged at 300g for 5 minutes. The supernatant was then discarded and the cell pellets were frozen at -80 ^{o}C for at least 3-4 hours to aid cell lysis.

2.3.8 Reverse Transcription Polymerase Chain Reaction

Reverse Transcription polymerase chain reaction (RT-PCR) was performed on cell lysates obtained from HEK-293 cells transfected with wildtype N1 and either wildtype or mutant N2A constructs, to compare wildtype and mutant N2A gene expression.

2.3.8.1 RNA extraction

Ribonucleic acid (RNA) was extracted from cell pellets collected after 24 hours of transfection with N1 wildtype and N2A wildtype or mutant constructs. This procedure was performed using The RNeasy Mini Kit (Qiagen) using the manufacturer's instructions.

Briefly, working under a fume hood, the cell pellet was disrupted by adding 350µl of Buffer RLT with 1% β-mercaptoethanol and mixing thoroughly. The suspension was

vortexed for 1 minute then 350µl of 70% ethanol was added. The suspension was again mixed thoroughly by pipetting up and down repeatedly. The 700µl sample was then transferred to an RNeasy spin column placed in a 2ml collection tube and centrifuged for 15 seconds at ≥8000 x *g*. The flow through was discarded and 700µl of Buffer RW1 was added to the spin column before the sample was centrifuged again at ≥8000 x *g* for 15 seconds, and the flow-through discarded. Following this, 500µl of Buffer RPE was added, the sample was centrifuged at ≥8000 x *g* for 15 seconds and the flow-through was discarded. This last step with Buffer RPE was then repeated with a longer centrifugation time of 2 minutes to wash the spin column membrane. After this centrifugation, the RNeasy spin column was placed in a new 2ml collection tube and centrifuged one more time at ≥8000 x *g* for 1 minute to eliminate any possible carryover of Buffer RPE, before being placed in another new 1.5 ml collection tube. 30µl of RNase-water was then added directly to the spin column membrane and RNA was eluted by centrifuging for 1 minute at ≥8000 x *g.* The eluted RNA was measured using NanoDrop, then used directly for reverse transcription.

2.3.8.2 Purification and Reverse Transcription

Purification and reverse transcription of RNA was carried out using the QuantiTect Reverse Transcription Kit (Qiagen).

Genomic DNA wipeout reactions were prepared in ice in 14µl reactions as follows:-

gDNA Wipeout Buffer, 7x	2µl
Template RNA	1µg
RNase-free water	to make up volume to 14µl

Each reaction was incubated at 42°C for 2 minutes before being placed back on ice.

Reverse transcription master-mix was prepared in ice in 20µl reactions as follows:-

Quantiscript Reverse Transcription buffer 5 x	4µl
Reverse transcription primer mix	1µl
Quantiscript Reverse transcriptase	1µl
Template RNA (genomic wipeout reaction)	14µl

The reverse transcription reactions were incubated at 42°C for 15 minutes then at 95°C for 3 minutes to inactivate the reverse transcriptase, before being placed back on ice.

The resultant cDNA obtained was then diluted 1:50 in RNAse free water before proceeding to real-time PCR amplification.

2.3.8.3 Real-Time PCR Amplification and quantification

Real-time PCR was carried out in 20µl reactions for each target gene (*GRIN2A)* and reference gene (GAPDH) as follows:-

Mesa Blue PCR mastermix 2x	10 µl
cDNA Primer mix	1µl of 10µM concentration
cDNA from Reverse Transcription	9µl

Each reaction was carried out in triplicate on a skirted 96-well plate. cDNA primer sequences are presented in **Table 2-5**.

Table 2-5 cDNA Primer Sequences

Gene	**cDNA Primer Pair**
GRIN2A	Forward: 5'-caatgggaagcatggcaaga-3' Reverse: 5'-gctagccaggaatatgacagc-3'
GAPDH	Forward: 5'-ttgaggtcaatgaaggggtc-3' Reverse: 5'-gaaggtgaaggtcggagtca-3'

Reactions were run on the Applied Biosystems StepOne Real-Time PCR System (ThermoFisher), and results were exported into a Microsoft Excel spreadsheet for analysis.

2.3.8.4 Analysis of results

To quantify gene expression, the cycle of threshold (CT) for each gene transcript was determined. CT is the number of cycles of PCR amplification at which detected fluorescence from the PCR product rises above a set threshold. Relative gene expression was calculated for each *GRIN2A* mutation in relation to wild-type using the delta-delta CT (ΔΔCT) method described by Schmittgen and Livak (Schmittgen and Livak, 2008).

RE=2^(-ΔΔCT)

ΔΔCT = ΔCT sample (target-reference)- ΔCT control (target- reference) or in this case;

ΔΔCT = ΔCT R518C/R518H (*GRIN2A* - GAPDH)- ΔCT wild-type (*GRIN2A* - GAPDH)

Mean and standard deviation of relative expression values were obtained through triplicate measurements for each genotype. One- way Anova was performed in Prism 7.0 (Graph Pad) to compare the difference in gene expression between R518C/R518H transfected and wild-type N2A transfected cells.

2.3.9 Western Blotting

Immunoblotting was performed on cell lysates obtained from HEK-293 cells that I transfected with wildtype N1 and either wildtype or mutant N2A constructs, to compare wildtype and mutant N2A protein expression.

2.3.9.1 Cell lysis

Lysis buffer for each sample was prepared on ice as follows:-

RIPA buffer	180µl
Pierce Protease and Phosphatase inhibitor (1 tablet dissolved in 1ml of dH_2O)	20µl

Each cell pellet was re-suspended in 200µl of lysis buffer on ice then incubated on a roller at 4^{o}C for 30 minutes. Following this, the samples were centrifuged at 13,200rpm for 15 minutes at 4^{o}C. The cell lysates (supernatant) were then decanted and stored at -20 ^{o}C.

2.3.9.2 Measuring protein concentration of cell lysates

The Pierce BCA Protein Assay kit was used to measure the total protein concentration of cell lysates prior to Western blotting

Pierce pre-diluted bovine serum albumin (BSA) standards (ThermoFisher) with protein concentrations ranging from 125µg/ml to 2000µg/ml were used as reference samples.

10µl of pre-diluted BSA standards and 10µl of each cell-lysate to be measured was added to each appropriately- labelled well in a flat-bottomed 96- well plate. 2 samples of each pre-diluted BSA standard, and 3 samples of each cell-lysate were included on each plate.

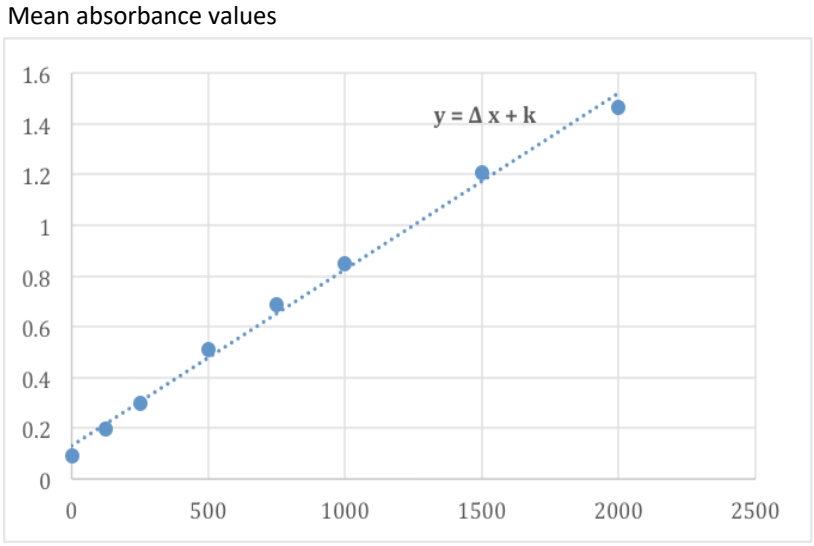
200µl of BCA working solution was prepared for each sample by adding BCA Reagent A to BCA Reagent B in a ratio of 50:1. 200µl of working solution was then added to each sample on the 96-well plate.

Following this the 96-well plate was covered and briefly shaken (approximately 30 seconds) on a plate shaker before incubation at 37°C for 30 minutes.

After incubation, the colorimetric absorbance of each sample on the plate was measured using Tecan Infinite 200 PRO multimode plate reader, with the absorbance wavelength set at 555nm, bandwidth 10nm. Results were recorded using Tecan i-control 1.12 software.

The mean absorbance value of the 2 samples of each pre-diluted BSA standard on the plate was then calculated and used to plot a linear graph, with the known protein concentration of the standards on the X-axis and their mean absorbance values on the y-axis **(Figure 2-3**).

Figure 2-3 Linear graph of known protein concentration vs mean absorbance value



The total protein concentration of each cell-lysate was then derived using the following formula:-

Protein concentration = (M - k)/ Δ

(M = mean of the absorbance values for the 3 samples of each cell-lysate loaded; k = y-intercept of graph obtained; Δ = gradient of linear graph obtained).

2.3.9.3 Gel loading, electrophoresis and protein transfer

Cell-lysate protein samples (10µg/well) were prepared in 4 x Laemmli buffer (Bio-Rad) and 5 x Dithiothreitol (DTT) (ThermoFisher).

The samples were then heated at 37 °C for 15 min and spun down briefly before loading onto appropriate wells on 4-20% Mini-Protean TGX Stain-Free Pre-cast gels (Bio-Rad). 5µl of Precision plus dual colour standards marker (Bio-Rad) was loaded into the first lane for each run.

Gels were immersed in 1x Tris-Glycine SDS (sodium dodecyl sulphate) buffer in a BioRad electrophoresis chamber and ran at 200V for 50 minutes.

Proteins were then transferred onto a polyvinylidene difluoride (PVDF) membrane from the Trans-blot Turbo Mini PVDF transfer pack (Bio-Rad), using the Trans-Blot Transfer System.

2.3.9.4 Immunodetection

After protein transfer, the PVDF membrane was cut at the 50kDa marker. The top half (>50kDa, Part A) was incubated at room temperature for 60 minutes in a blocking solution made up of 10% skimmed milk in phosphate buffered saline (PBS) with 0.1% Tween-20. It was then incubated at 4^{o}C overnight with 1:1,000 N2A antibody (M264, Sigma-Aldrich) in 5% skimmed milk, PBS and 0.1% Tween-20.

The bottom half (<50kDa, Part B) was incubated at room temperature for 60 minutes in a blocking solution made up of 5% skimmed milk in PBS with 0.5% Tween-20. It was then incubated at 4^{o}C overnight with an antibody acting as a loading control - 1:5,000

GAPDH antibody conjugated with horse radish peroxidase (HRP) (Cell Signalling Technology), in PBS with 0.5% Tween-20.

The following day, Part A (>50kDa) was washed in PBS with 0.1% Tween-20 for 10 minutes, three times, before incubation for 1 hour at room temperature with 1:10,000 anti-rabbit HRP conjugated secondary antibodies. As the GAPDH antibody used for Part B (<50kDa) was already conjugated with HRP, this did not need incubation with secondary antibodies the following day. Part B was simply washed in PBS with 0.5% Tween-20 for 10 minutes, three times.

Following these washes, Clarity Western ECL Substrate solution was prepared by mixing the two substrate kit components in 1:1 ratio. 10ml of substrate solution was prepared for each part of the membrane. Both Part A and Part B were incubated in this substrate solution for 5 minutes, before protein bands were imaged using the ChemiDoc™ MP Imaging System (Biorad). Results were analysed using ImageJ (https://imagej.nih.gov/ij/). The density of each protein band was measured using ImageJ and normalized to GAPDH. The result for each mutation was then compared to that of wild-type to obtain relative protein expression for R518C and R518H.

$$\text{A.R.D} = \frac{[\text{N2A density (R518C or R518H)} \div \text{N2A density WT}]}{[\text{GAPDH density (R518C or R518H)} \div \text{GAPDH density WT}]}$$

[A.R.D.: Adjusted relative density, WT: wild-type]

Mean and standard deviation values were obtained through measurements from three independent transfections. One-way Anova was performed in Prism 7.0 (Graph Pad) to compare the difference in protein expression between each mutation and wild-type N2A transfected cells.

2.3.10 Immunocytochemistry

Immunocytochemistry experiments were performed on HEK-293 cells transfected with wildtype N1 and either wildtype or mutant N2A constructs, to compare membrane surface expression of wildtype and mutant N2A receptors.

2.3.10.1 Cell Fixation

24 hours after transfection, cell culture medium was aspirated and replaced with 1% paraformaldehyde (PFA) in complete media. The cells were then incubated at room temperature for 20 minutes. Following this, the cells were rinsed three times with 1 x phosphate buffered saline (PBS), then fixed by incubation with 4% PFA in 1x PBS, for 20 minutes at room temperature. After fixation, the cells were rinsed for a further three times with 1x PBS before proceeding to immunostaining.

2.3.10.2 Immunostaining and quantification

In order to determine N2A surface expression, the cells were immunostained without being permeabilised. For immunostaining, the cells were first incubated in blocking buffer (1 x PBS with 10% FBS) for 30 minutes at room temperature. After 30 minutes, the blocking buffer was discarded and the cells were incubated overnight at 4^{o}C with 1:1000 Anti-GRIN2A extracellular antibody (Alomone Labs) in blocking buffer solution (1 x PBS with 10% FBS).

The following day, the cells were washed thrice in 1 x PBS then incubated for 45 minutes at room temperature with 1:800 Alexa-Fluor Goat Anti-rabbit 594nm IgG secondary antibody (Abcam) and 1:1000 CF™488A wheat germ agglutinin (Biotium) in 1 x PBS with Ca^{2+} and 10% FBS.

After 45 minutes, the cells were washed twice with 1 x PBS, then incubated with 1:1000 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) in 1 x PBS with Ca^{2+} for 5 minutes in room temperature. Following this the cells were rinsed once more with 1 x PBS before the slide was mounted using Prolong Gold Anti-fade reagent (ThermoFisher).

The slides were viewed on the Zeiss LSM 710 inverted confocal microscope and images were analysed using Fiji software (https://fiji.sc/).

2.3.10.3 Analysis

Z-stack images were taken at x63 magnification on the Zeiss LSM 710 inverted confocal microscope. 5 images were taken for each genotype yielding an average of approximately 100 nuclei per genotype. These images were then analysed using a

macro for Fiji software, written and provided by Dr Dale Moulding, UCL-Great Ormond Street Institute of Child Health. Using this macro, the intensity of N2A immunofluorescence co-localizing with the membrane surface marker, wheat germ agglutinin (WGA) was measured and averaged to the number of nuclei counted in the image. As the macro was designed to analyse all the images without manual input, I was blinded to the outcome until the analysis was completed.

The results obtained for R518C/R518H transfected cells were compared to the results obtained for wild-type transfected cells to obtain relative surface-expression values. Mean and standard deviation of these relative expression values were obtained through repeating measurements for three independent transfections. One-way Anova was performed in Prism 7.0 (Graph Pad) to compare the difference in surface expression between R518C/R518H transfected and wild-type N2A transfected cells.

2.3.11 Homology Modeling

Through an established lab collaboration, Dr Sony Malhotra (Department of Biochemistry, University of Cambridge) and Dr Maya Topf (Institute of Structural and Molecular Biology, UCL Birkbeck College) carried out homology modelling to determine the structure-function properties of the *GRIN2A* missense variants identified in this cohort. This was performed using the crystal structure available for the ligand-binding domain of N2A in the protein structure databank (PDB), 5H8F (glutamate bound heterodimer of N2A and N1), solved at 1.81 Å (Hackos et al., 2016).

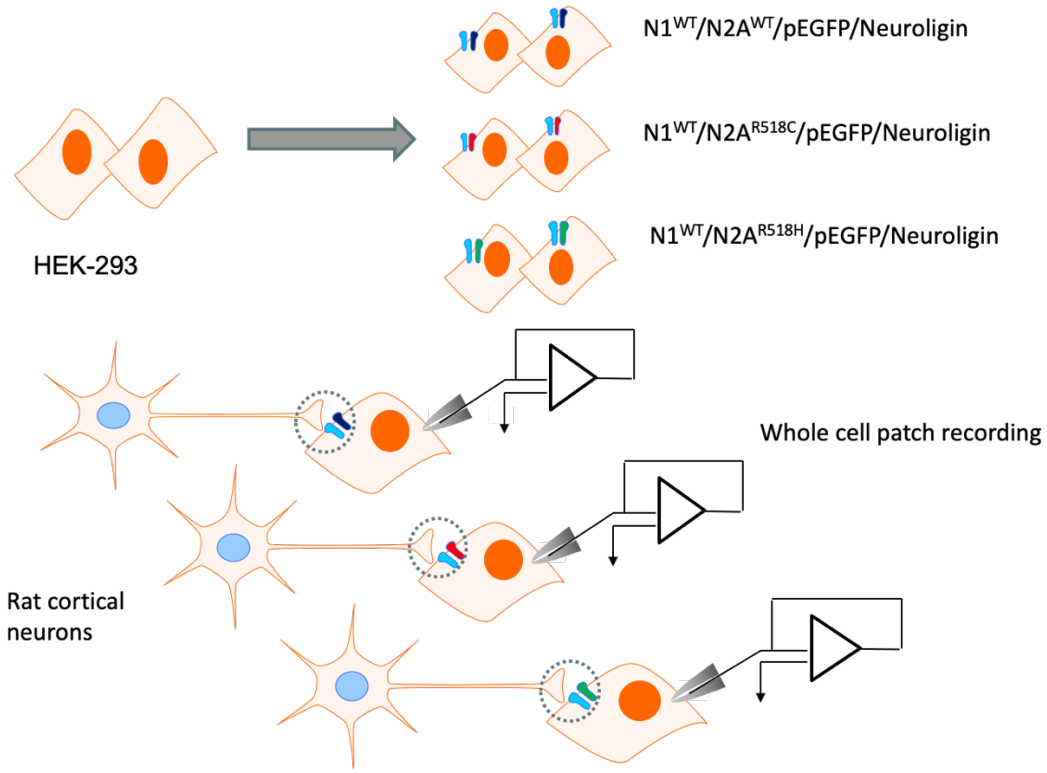
2.3.12 Electrophysiology

In order to determine the effect of the identified *GRIN2A* missense variants on channel function, electrophysiology experiments were performed externally by our collaborators, Professor Robert Harvey and Dr Joe Lynch at the University of the Sunshine Coast, Queensland Australia.

Rat cortical neurons and HEK-293 cells transfected with either wild-type or mutant *GRIN2A* were co-cultured to create 'artificial' synapse preparations (Dixon et al., 2015). HEK-293 cells were transfected with N1-WT, N2A-WT or N2A- mutant (R518C/R518H) plasmids, pEGFP and mouse neuroligin at ratio of 1:1:1:0.5:1, using a calcium-phosphate

co-precipitation protocol. These were plated onto rat cortical neurons that had been allowed to culture and grow for 3-4 weeks. Artificial synapses formed spontaneously after 24 hours and whole-cell patch clamping at a holding potential of -70mV was performed at room temperature, to record excitatory post-synaptic currents (EPSCs), 2-5 days later (**Figure 2-4**).

Figure 2-4 Electrophysiology



HEK-293 cells were co-transfected with N1-WT, one of N2A-WT, N2A-Arg518Cys or N2A-Arg518His plasmids, pEGFP and mouse-neuroligin using a calcium phosphate co-precipitation protocol. These cells were then co-plated with cultured rat cortical neurons to form artificial synapses for whole cell patch clamp recording.

3 Chapter 3: Clinical Characterization of the LKS Cohort

3.1 Introduction

In this part of my PhD project, I carried out deep clinical endophenotyping of the recruited cohort to (i) better characterize clinical features of Landau Kleffner Syndrome (LKS), (ii) determine what factors may influence outcome, and (iii) define phenotypic features associated with specific genetic sub-groups.

Reflecting the rarity of LKS, this is to my knowledge, one of the largest LKS cohort studies, with one of the longest follow-up periods, thereby providing more robust data for statistical analysis.

3.2 The Great Ormond Street Hospital LKS Cohort: case ascertainment

From January 1990 to June 2018, a total of 95 children were referred to the Developmental Epilepsy Clinic (DEC) at Great Ormond Street Hospital (GOSH) for assessment of Landau Kleffner Syndrome (LKS). Of these, 91 consented to participating in this research study.

Due to limited time available for this study, phenotypic data was collected for a total of 70 patients. The study started with random selection of patients for collection of phenotypic data. However, as the study progressed, I prioritized collection of data from patients for whom DNA was available, and patients who I had the opportunity to review personally in clinic. Of the 70 patients studied, DNA was available for 62 patients (**Figure 3-1**). I had the opportunity to meet and collect data from 24 patients.

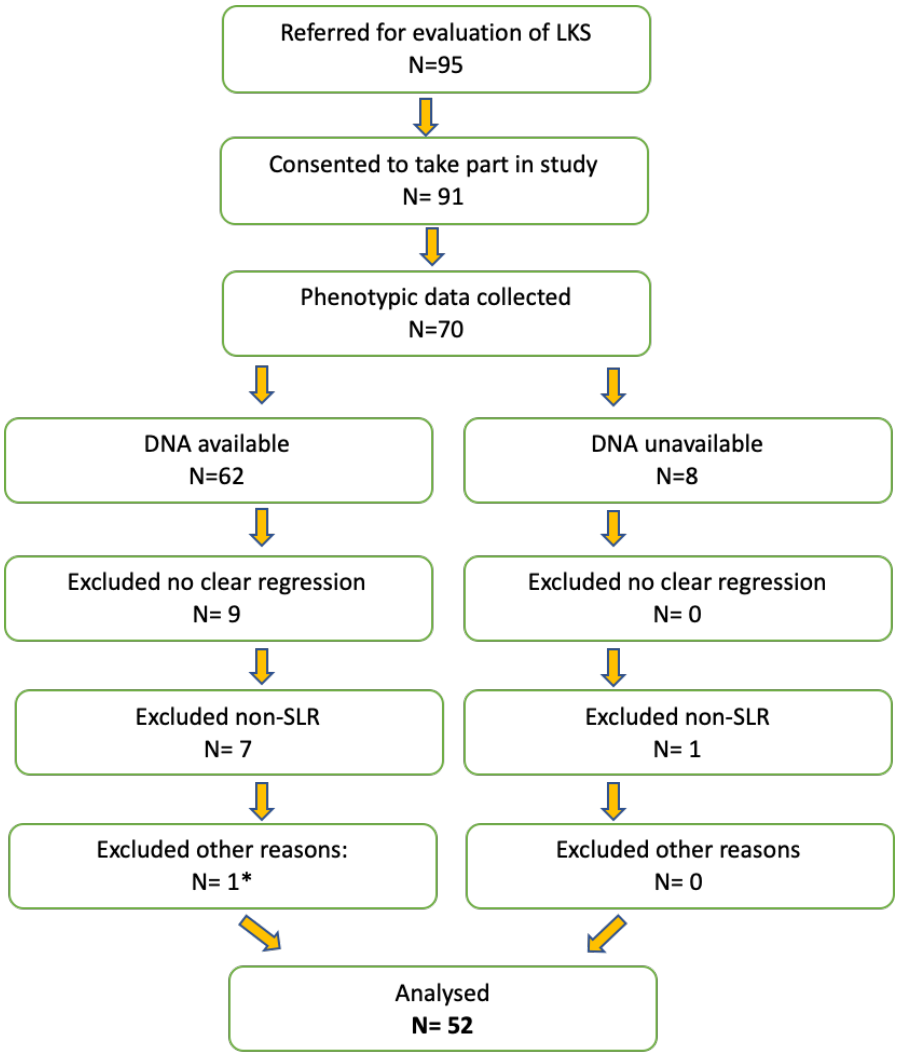
All patients included in the study met the inclusion criteria of (i) predominant speech and language regression and (ii) a consistent seizure disorder characterized by seizures and/or EEG findings of either ESES or focal centro-temporal discharges, which activate in sleep. Patients who had long-standing speech and language impairment without a clear history of regression (n=9) and patients who had clinical features more in keeping with ECSWS, with a more global or non-speech and language regression phenotype (n=8), were excluded. 1 patient was later discovered to be positive for N-methyl-D-

aspartate receptor antibodies and was excluded. This left 52 patients for analysis (**Figure 3-1**).

Collected data includes information regarding: (i) current age, (ii) gender, (iii) age at onset of speech and language regression, (iv) the presence or absence of seizures, (v) if relevant, the age of onset of seizures, (vi) seizure semiology, (vii) EEG findings, (viii) prior speech and language/other developmental delay, (ix) non-verbal skills, (x) behavioural difficulties, (xi) motor difficulties, (xii) treatments received (xiii) seizure/language outcome at last assessment, (xiv) family history and (xv) if current age ≥18 years, level of independence and language ability as an adult.

Table 3-1 presents data collected for these 52 patients in comparison to data collected from other reported cohort studies.

Figure 3-1: Number of patients included in LKS clinical cohort study



N: number of patients; SLR: speech and language regression; *NMDA receptor antibody positive.

Table 3-1 Data collected for LKS cohort study in comparison with previously reported cohort studies

Study	(Mantovani and Landau, 1980)	(Deonna et al., 1989)	(Soprano et al., 1994)	(Rossi et al., 1999)	(Duran et al., 2009)	(Robinson et al., 2001)	(Cockerell et al., 2011)	(Caraballo et al., 2014)	Present Study
N.	9	7	12	11	7	17	19	29	52
Male	33.3%	85.7%	75%	63.6%	100%	35%	NR	68.9%	59.6% ± 13.3%[§]
Mean length of FU (range)	21y9m (10y to 28y)	18y6m (13y to 27y6m)	8y11m (2y6m to 15y4m)	9y8m (2y3m to 16y2m)	13y3m (5y to 19y)	5y 7m (1y to 15y)	10y9m (1m to 25y)	12y (3-21 y)	14y3m ± 7y11m[II] (3y2m to 29y1m)
Positive FH	22.2%	NR	NR	45.5%	NR	11.7%	NR	NR	42.3% ± 13.4%[§]
Pre-existing SD	0%	14.2%	75%	27.3%	NR	23.5%	0%	65.5%	28.8% ± 12.3%[§]
Mean age at SLR (range)	5y9m (3y to 9y)	5y (3y to 8y)	4y10m (4y to 5y6m)	3y5m (1y6m to 5y8m)	4y6m (3y to 9y)	4y8m (2y1m to 7y)	3y7m (1y5m to 6y)	5y (2y to 9y)	4y9m ± 2y[II] (1y6m to 11y10m)
Clinical Sz	66.6%	85.7%	58%	81.8%	85.7%	88.2%	47%	79.4%	84.6% ± 9.8%[§]
ESES on EEG (SA)	NR	NR	NR	100%	28.6% (71.4%)	NR (100%)	NR (89.5%)	51.7% (41.3%)	55.8% ± 13.4%[§] (25.0% ± 11.8%[§])[†]
< average NVS	0%	28.6%	50%	81.8%	NR	11.8%	15.7%	48.3%	46.2% ± 13.6%[§]
BD at presentation	44.4% (HA/Agg)	71.4% (Agg)	75% (HA)	72.7% (> 1: Agg, ASD, ADHD, HA)	42.8% at last FU (HA, ASD)	52.9% (Agg/ ASD, HA)	89.5% or > (Agg, ADHD, HA)	65.5% (ADHD, Agg,)	76.9% ± 11.5%[§] (HA, ADHD, Agg, ASD)
Language OC at last FU	NL: 44.4% MI: 11.1% MDI: 44.4%	NL: 28.6% MI/MDI: 14.3% SI/NS: 42.9%	NL: 25% MI/MDI:57.9% SI: 16.6%	NL: 18.2% MDI: 45.5% SI: 36.3%	NL: 14.3% MI- SI: 85.8% (42.9% PR)	NL: 17.6% MDI: 23.5% SI/NS: 23.5%*	NL: 21% MDI: 58% SI/NS: 21%	NL: 27.5% MI-SI:73.5%	NL: 26.9% ± 12.1%[§] MI: 15.4% ± 9.8%[§] MDI: 19.2% ± 10.7%[§] SI: 23.1% ± 11.5%[§] NS: 15.4% ± 9.8%[§]
Sz at last FU	0%	14.3%	Not reported	0%	28.6%	Not reported	0%	10.3%	21.2% ± 11.1%[§]
Age at SLR corr with language OC	No	No	No	Early onset corr with worse OC	Late onset corr with worse OC	No	<5y a/w worse OC (p=0.02)	Early onset corr with worse OC	Early onset corr with worse OC

Study	(Mantovani and Landau, 1980)	(Deonna et al., 1989)	(Soprano et al., 1994)	(Rossi et al., 1999)	(Duran et al., 2009)	(Robinson et al., 2001)	(Cockerell et al., 2011)	(Caraballo et al., 2014)	Present Study
Outcome at >18y	N: 7 NL/I: 57.1% MI/I 14.3% MDI/I: 28.6%	N.: 7 I: 42.9% D: 57.1%	N: 3 NL: 66.6% MDI: 33.3% I: NR	N.: 1 SI: 100% I: NR	N.: 4 NL/I: 25% MI – SI/D: 75% (25% PR)	N.: 0	N.: 4 NL/I: 75% MDI/I: 25%	N.: 13 NL/I: 46.2% MI-MDI/I: 30.8% SI/D: 23.1%	N: 25[‡] NL-MI/I: 56%±19.5%[§] MDI-SI/I: 28% ± 17.6%[§] D: 16% ± 14.4%[§]

ADHD: attention deficit hyperactivity disorder; Agg: aggression; ASD: autistic traits; a/w: associated with; BD: behavioural disorder; corr: correlated; D: dependent; ESES: electrical status epilepticus in sleep (85% of spike wave index); FH: family history of seizures or speech and language impairment; FU: follow-up; m: months; HA: hyperactivity; I: independent; MI: mild speech impairment; MDI: moderate speech impairment; N.: number of patients; NL: normal language; NR: not reported; NS: no speech; NVS: non-verbal skills; OC: outcome; PR: partial remission; SA: sleep activation (<85% spike wave index); SD: speech delay; SI: severe speech impairment; SLR: speech and language regression; Sz: seizures; y: years
*remainder: not reported; [†]9/52 (17.3%) did not have sleep EEG data available; [‡] formal speech and language assessments were not repeated; [§]95% confidence interval; [ll]Standard deviation

3.3 Clinical Features

The mean current age of patients within the LKS cohort ranged from 7 years 3 months to 33 years 9 months. Clinical features have been summarized in **Table 3-1**, alongside findings from previous cohort studies. These are compared in more detail below.

3.3.1 Patient gender and age of disease onset

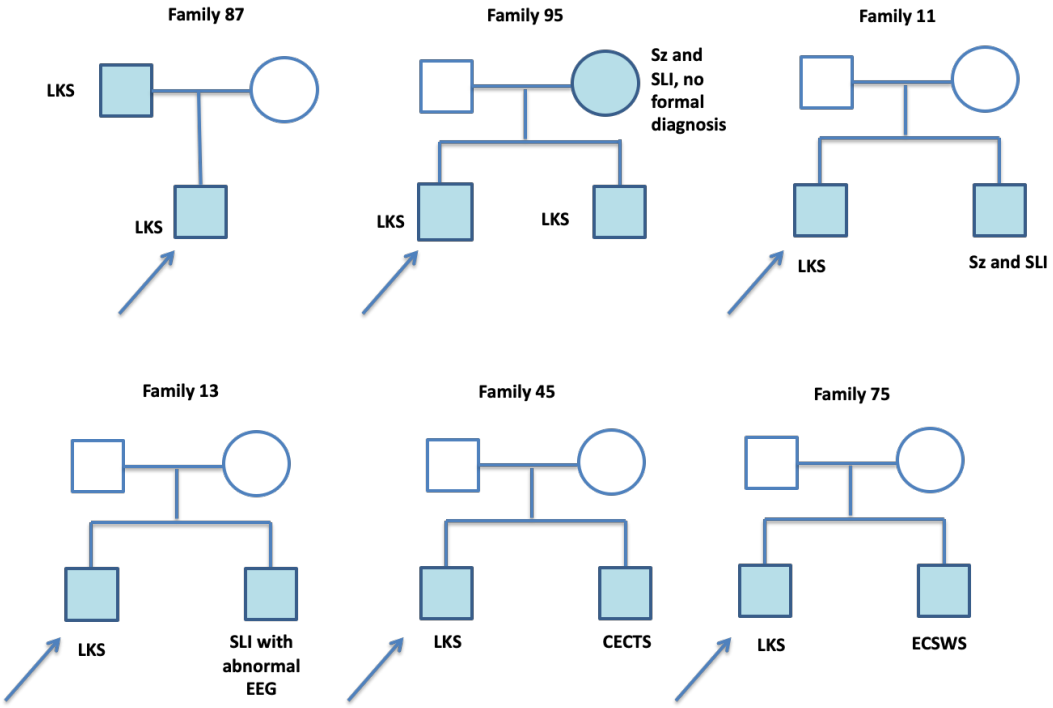
59.6% of patients in the GOSH LKS cohort are male. Some earlier (smaller) studies have reported a male preponderance while others have reported the opposite (**Table 3-1**).

The age of onset of aphasia has been reliably uniform across all cohort studies over the last nearly 4 decades, with a mean age of presentation centring around 3.5-5.5 years of age. As mentioned in Chapter 1, this age predilection supports the hypothesis that LKS is likely to be a developmental disorder.

3.3.2 Family History

Nearly half the patients in our LKS cohort, (42.3%), have a positive family history defined for the purposes of this study, as a history of epilepsy, and/or speech and language difficulties in a first or second-degree relative (**Table 3-1**). Using these criteria, the GOSH LKS cohort includes 6 families with either parent-child or sibling pairs with similar (but not always identical) presentations **(Figure 3-2).** This is compelling evidence that genetic factors are likely to have a role in the pathogenesis of this disorder.

Figure 3-2 Examples of familial cases in GOSH LKS Cohort



Ax: assessment; CECTS: childhood epilepsy with centrotemporal spikes; ECSWS: epilepsy syndrome with continuous spike waves in sleep; LKS: Landau Kleffner syndrome, SLI: speech and language impairment; Sz: seizure *For Family 75, the older brother had classical LKS; he consented for the study of his case history but not for blood sampling and DNA extraction. The younger brother's DNA was available, but he did not have classical LKS so he was excluded from the LKS clinical cohort study. The older brother's case history was used for the purpose of the LKS cohort study, but the younger brother's DNA was sent for triome whole genome sequencing.

3.3.3 Epileptic seizures and ESES

In the GOSH LKS cohort, 84.6% of patients had epileptic seizures, compared to 47% to 88.2% in other cohorts **(Table 3-1).** The most frequently reported seizure type in our cohort was focal motor seizures occurring from sleep. This is consistent with previous reports (Caraballo et al., 2014, Duran et al., 2009, Rossi et al., 1999). Other seizure types include absence seizures, atypical absences, myoclonic, atonic and generalized tonic-clonic seizures.

The activation of epileptic discharges in sleep was not widely recognized until the 1980s, and therefore some of the earlier cohort studies have not reported on ESES or other EEG findings in sleep. Furthermore, there is considerable variation in the proportion of ESES patients reported in later studies, which appears to reflect differing expert opinions on

the definition of ESES. When Patry et al first described ESES, it was arbitrarily defined as a spike wave index (the percentage of non-REM sleep occupied by spike-wave discharges) of ≥85% (Patry et al., 1971). However, others have later proposed that activation of discharges in sleep is significant even at spike-wave indices (SWI) below 85% (Nickels and Wirrell, 2008). For the purpose of this study, I have simply kept to Patry's original definition. Patients were regarded as having ESES if their EEG reports specifically indicated "continuous epileptiform activity in sleep", or if their spike-wave index was 85% or greater.

For all studies, nearly 100% of patients had either ESES or sleep activation on EEG.

55.7% of our cohort had ESES at some point in their disease course. A further 23.1% had evidence of sleep activation with a SWI of 30-81%. Some of our patients 9/52 (17.3%), mostly older patients who had language regression in the 1980s, did not have sleep EEG recordings available.

3.3.4 Pre-existing speech delay, non-verbal deficits

The International Classification of Disease, ICD-10 criteria for acquired epileptic aphasia or Landau Kleffner Syndrome follows the original description by Landau and Kleffner closely (Stefanatos, 2011). It deems "previously normal progress in language development" and "preservation of general intelligence and hearing" as necessary criteria for LKS diagnosis.

Notwithstanding this, many previous cohort studies have included patients with pre-existing speech and language delay and non-verbal cognitive impairment amongst their LKS patients on the basis of other overlapping electroclinical features. The resulting inconsistencies observed in different studies can thus be attributed to differing opinions regarding the presence and severity of prior speech and cognitive impairment in true LKS. For instance, Cockerell et al (2011) excluded all patients with prior speech impairment, while Robinson et al (2001) only excluded patients who did not meet the criteria of "single words by 2 years and joining words by 3 years" (**Table 3-1**). Having reflected upon these observations, for this study I have decided to include patients with prior delay in speech development, but only if there is evidence of clear regression of previously gained skills. Furthermore, I have included patients with pre-existing or

subsequent impairment in non-verbal skills, only if speech and language regression was the overriding dominant feature at presentation. As such, 28.8% of our cohort had pre-existing speech delay. 44.2% of this cohort had below average non-verbal skills ranging from mild impairment to severe impairment.

3.3.5 Behavioural difficulties

It is evident from all the previous studies that behavioural disorders are common in LKS. In our study, the strengths and difficulties questionnaire filled out by parents revealed behavioural concerns in 76.9% of patients (**Table 3-1**). Aggression is most commonly reported (54%), followed by hyperactivity (38%). Other behavioural difficulties included attention deficits, autistic traits, mood swings, anxiety and obsessive-compulsive traits, and self-harming (see **Appendix** for clinical summaries of some patients).

Parents commonly report uncharacteristic aggressive rage/tantrums and impulsive, oppositional behaviour. These behavioural difficulties are often severe and extremely difficult to manage, having a significant impact on a family's quality of life. A pair of parents reported that they felt like they had lost their child and that the child they now had was not the child they once knew.

For some children, behavioural disorders were exacerbated by steroid therapy, and therapy had to be discontinued. Some families benefited from referral to clinical psychology for behavioural management strategies and/or referral for respite care. A few children were helped by pharmacological treatment for attention deficit hyperactivity disorder **(**see **Appendix** for clinical summaries of some patients**).**

3.3.6 Motor difficulties

Motor difficulties have rarely been reported in previous cohort studies of Landau Kleffner Syndrome. However, I found that about a third of our LKS patients (28.8%) had motor difficulties. For the majority, these were fine motor deficits, coordination difficulties and dyspraxia, leading to difficulties in hand-writing, managing buttons and feeding. Some patients were noted to have an awkward, clumsy gait with frequent falls and difficulty managing stairs, usually due to problems with motor coordination or balance. A few were noted to have a fine hand tremor on examination **(Appendix)**.

For 1 patient, motor difficulties (dyskinesia and tremor) were noted at presentation before initiation of medication. For others, motor difficulties were noted during the course of their illness, and it is not possible to tell if these are due to progression of disease or if they may be due to effects of medication. For many patients, it is also difficult to be sure if motor difficulties were not already present at the time of diagnosis because GOSH, being a tertiary hospital, is not their first port of call; parents and referral letters may not highlight motor difficulties, and it is possible that some subtle motor difficulties may have been over-shadowed by the more alarming symptoms of seizures and acquired aphasia.

Motor difficulties often improved along with improvement in seizure burden and language, however, many patients had mild residual difficulties with fine-motor skills and or motor-coordination. Some patients benefited from referral for occupational therapy and/or physiotherapy.

3.4 Outcomes

3.4.1 Language Outcomes

It is not easy to compare language outcomes across different cohort studies as the way language outcomes were classified varied greatly among different studies. Furthermore, not all studies have clarified how they have defined the categories selected for assessing language outcome.

26.9% (95% confidence interval: 14.8% to 39%) of our cohort had recovered language at their last assessment, scoring within the average range expected for their age compared to 14.3% to 44.4% in previous studies. The remaining had some degree of language impairment ranging from mild language impairment to no functional language at last follow up. For this study, the definitions of these categories for language outcomes were presented in **Chapter 2, Section 2.2.2.** It is difficult to compare the proportion of patients with mild to severe language outcomes across the different studies, as each study had different definitions for these categories.

15.4% (95% confidence interval: 5.6% to 25.2%) of our cohort had mild impairment in language, 19.2% (95% confidence interval: 8.5% to 29.9%) moderate impairment, 23.1% (95% confidence interval: 11.6% to 34.6%) severe impairment, and 15.4% (95% confidence interval (95% confidence interval: 5.6% to 25.2%) had no functional language at last follow up.

Examining language outcomes in our LKS cohort (despite its wide age range, and variable follow-up period) is useful as a rough indication of the percentage of individuals who respond to treatment during the course of illness. However, an important caveat is that this does not take into consideration the well-recognized fact that a significant proportion of LKS patients have a relapsing- remitting course during childhood and adolescence. The disorder is thought to stabilize in adolescence (Tuft et al., 2015). As such, it may be more accurate to look at outcomes in adulthood.

3.4.2 Outcomes in Adulthood (> 18 years of age)

In 1980, Mantovani et al **(Table 3-1),** reported the long- term outcomes of 9 LKS patients including the first 6 patients described by Landau and Kleffner in 1957. It is interesting to note that every one of the 6 original patients, lived independently as adults. 3 were independent and reported to have normal speech and language. The other 3 were independent, despite residual mild to moderate speech and language impairment (Mantovani and Landau, 1980).

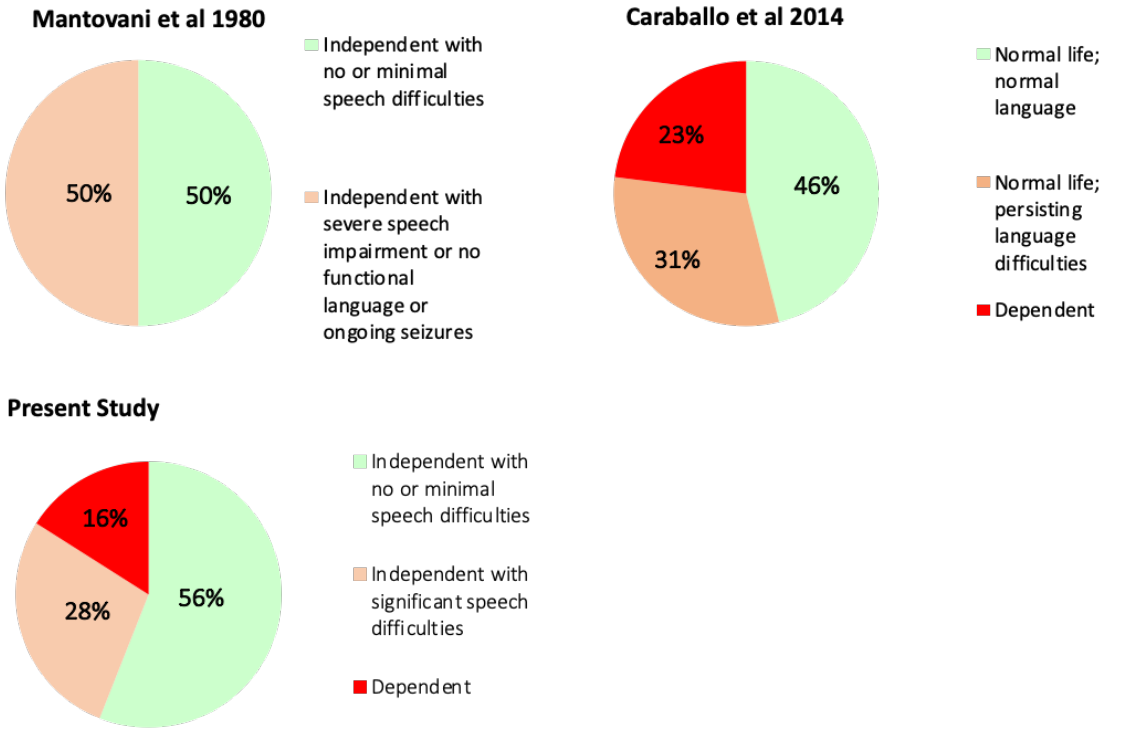
Since this study, other studies have reported that the proportion of individuals independent in adulthood (according to each study's authors' definition) range from 25% (Duran et al., 2009) to 77% (Caraballo et al., 2014). Most studies have reported that a proportion of individuals remain dependent in adulthood **(Table 3-1).**

In our cohort, 25/52 individuals are now adults (≥18 years of age). To my knowledge, this is the largest number of patients followed up into adulthood. These individuals had a variety of treatments during the course of their illness, including steroid therapy, immunoglobulin infusions and more invasive interventions such as multiple sub-pial transections. Categories for outcomes in adulthood were defined in **Section 2.2.2**, **Table 2-2**.

Today, 56% of these individuals are independent in employment or at university with no or mild language difficulties that do not impact significantly on their lifestyle. Examples of such mild language difficulties, include reports by family members that these individuals are too literal and do not understand speech nuances, or that they occasionally still have anxiety over speaking in public.

28% of the adult individuals in our cohort are independent but continue to have significant language difficulties; many of these individuals work and live within the deaf community, with sign language as their main means of communication. Finally, a further 16% continue to be dependent, with some living in residential care **(Figure 3-3).**

Figure 3-3: Outcomes in Adulthood



Reasons for ongoing dependence in adulthood include: (i) below average cognitive skills; (ii) persistent frequent seizures and (iii) difficulty forming relationships- either due to language difficulties or autistic traits.

The results from this study are comparable to results from Caraballo's recent study in 2014. Comparing these results, those of other recent studies and Mantovani's study **Table 3-1**, it is possible that there has been no significant improvement in reported long-term outcomes for children with LKS, since the first description of the disorder in 1957**.** However, it should be noted that the numbers in these studies are too small to draw any meaningful conclusion with certainty.

3.5 Factors Influencing Language Outcomes

To determine what factors may influence language outcome, I analysed collected data variables across the different language outcome groups. This data is presented in **Table 3-2**.

As mentioned in **Section 2.2.1,** the statistical programme, SPSS was used to derive statistical values including, frequency, mean values, standard deviation and range. The statistical test, one way analysis of variance (ANOVA) with Bonferroni correction was used to compare mean values for continuous variables. Fisher's exact test was used to compare categorical variables. A *p*-value of <0.05 was designated as the cut-off for significance.

Table 3-2: Differences in collected variables among language outcome groups

	Normal language	**Mild impairment**	**Moderate impairment**	**Severe Impairment**	**No functional language**	**(*p* value)**
No.:	14 (26.9%)	8 (15.4%)	10 (19.2%)	12 (23.1%)	8 (15.4%)	
Mean current age (range)	18y8m ± 8y9m (7y6m to 31y) [§]	18y4m ± 6y2m (10y10m to 28y1m) [§]	17y0m ± 7y0m (7y3m to 28y2m) [§]	16y9m ± 8y0m (9y6m to 30y3m) [§]	21y9m ± 9y4m (9y8m to 33y9m) [§]	0.689*
Male%	50.0%	62.5%	90.0%	50.0%%	50.0%	0.270[†]
Positive FH	21.4%	37.5%	60.0%	50.0%	50.0%	0.362[†]
Mean age at SLR (range)	5y6m ± 1y6m (3y3m to 8y0m) [§]	6y6m ± 2y3m (4y8m to 11y10m) [§]	4y1m ± 2y5m (1y6m to 8y10m) [§]	4y7m ± 1y6m (2y6m to 7y7m) [§]	3y2m ± 1y4m (1y6m to 4y9m) [§]	**0.006***
Prior speech and language delay	21.4%	37.5%	50.0%	25.0%	12.5%	0.409[†]
Clinical Sz	92.9%	75.0%	80.0%	75.0%	100.0%	0.438[†]
ESES on EEG (No.) [‡]	80.0% (8/10)	62.5% (5/8)	66.7% (6/9)	66.7% (6/9)	57.1% (4/7)	0.886[†]
< average NVIQ	35.7%	25.0%	40.0%	83.3%	37.5%	0.058[†]
BD	71.4%	62.5%	60.0%	100.0%	87.5%	0.142[†]
MD	21.4%%	25.0%	40.0%	41.7%	12.5%	0.551[†]
Ongoing Sz	21.4%	12.5%	30.0%	25.0%	12.5%	0.880[†]
Ongoing ESES [‡]	10.0%	0.0%	11.1%	22.2%	28.6%	0.517[†]

BD: behavioural difficulties; MD: motor difficulties; ESES: electrical status epilepticus in slow wave sleep; FH= family history; No.= number of patients; NVIQ: non-verbal intelligence quotient; SLR: speech and language regression; Sz: seizure. *One-way Anova with Bonferroni correction; [†]Fisher's exact; [‡]those without available sleep EEG reports (9/52, 17.3%) were excluded; [§]standard deviation

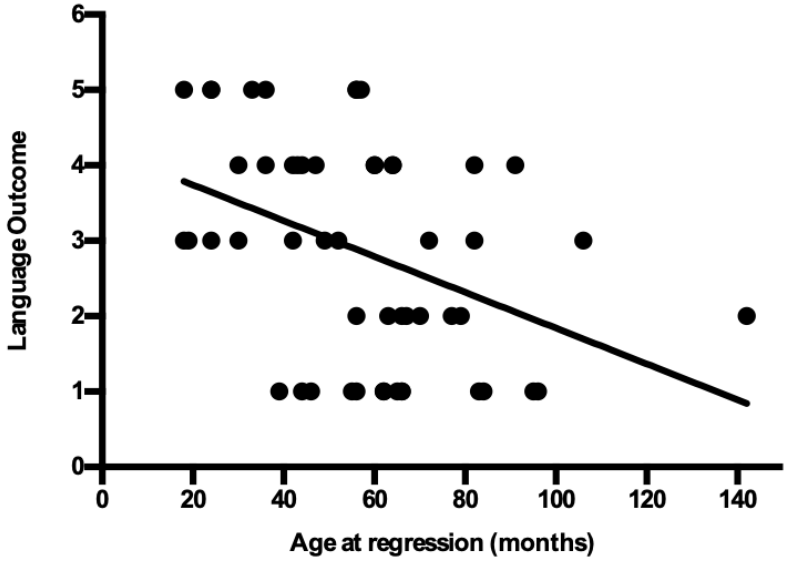
In my analysis, only 1 variable showed a statistically significant association with language outcome, namely, age at onset of language regression. 1 other variable, non-verbal IQ, approached statistical significance (*p*= 0.058).

3.5.1 Age at onset of speech and language regression and language outcome

Toso et al and Dulac et al in 1983 were one of the first to suggest that the age at onset of regression may be associated with language outcome. They both reported that regression onset before the age of 5 years had a negative impact on language outcome (Dulac et al., 1983, Toso et al., 1981). Some cohort studies have corroborated this association (Rossi et al., 1999, Cockerell et al., 2011), whilst other cohort studies have not (Robinson et al., 2001, Duran et al., 2009). In 1985, Bishop et al extended Dulac's research by investigating the relationship between age at onset of regression, and outcome in 61 previously reported cases of LKS from a variety of publications in the literature, followed up at least to the age of 12 years. They found a highly significant strong negative correlation between age at onset of regression and outcome (Spearman rho = -0.527, n=45, p<0.001) (Bishop, 1985). One limitation of Bishop's study is that the authors relied on case report descriptions to rate the degree of language impairment although they did acknowledge that this often proved difficult, as clinical descriptions were often vague. Such subjective categorization may lead to bias.

In my analysis, I also found a highly significant moderate negative correlation between age at onset of regression and language outcome (Spearman rho = -0.435, n=52, *p*=0.001; **Figure 3-4**). To my knowledge, this is the first single cohort study that has had enough patient numbers to perform such correlation analysis to statistical significance. Compared to Bishop's study, this study has the advantage of a more standardized categorization of language impairment (and thus a lower tendency for bias), as rating of language impairment was based on formal speech and language assessment results.

Figure 3-4: Correlation between age at regression and language outcomes. Spearman's Rho = -0.435, *p*= 0.001



Language outcomes grouped into: 1: Normal/Average language skills; 2: Mild impairment; 3: Moderate impairment; 4: Severe impairment; 5: No functional language.

3.5.2 Non-verbal skills impairment and language outcome

Not many studies have looked at the relationship between non-verbal cognitive impairment and language outcome. Robinson et al, (2001) found no correlation between language outcome and non-verbal IQ. However, they determined a borderline correlation between the length of ESES and subsequent non-verbal IQ (r=-0.43. p=0.081) and a significant correlation between length of ESES and both receptive (r=-0.73, p<0.001) and expressive (r=-0.67, p=0.007) language outcome.

In my study, there were more patients with below average non-verbal skills in the groups with poorer language outcomes – 83.3% of patients in the severe language impairment and 37.5% in the no functional language groups compared to 35.7% in the normal language outcome and 25.0% in the mild language impairment groups (**Table 3-2).**

It may be possible that children who are left with severe language impairment have a more severe disease process, which also renders them at greater risk for non-verbal cognitive impairment. Perhaps, for these children, their developmental disorder, seizures, language difficulties and cognitive impairment are all manifestations of a more abnormal developmental process.

It is important to note that for this analysis, it was not possible to reliably distinguish between pre-existing non-verbal deficits and non-verbal deficits that have occurred as part of the disease process. This is because most patients have not had any clinical need for pre-morbid non-verbal skills assessments.

3.5.3 Other factors linked to language outcome

Other factors that have been reported to influence language outcome include: (i) pre-existing speech delay (Rossi et al., 1999), (ii) the duration of ESES (Rossi et al., 1999, Robinson et al., 2001) (iii) the duration of aphasia (Rossi et al., 1999, Cockerell et al., 2011) and the presence of language fluctuation during the course of the disease (Cockerell et al., 2011, Deonna et al., 1977).

In my study, I found no significant correlation between pre-existing speech delay and language outcome.

As LKS can be a relapsing-remitting disorder, and as many of the patients in this cohort have not reached a stable phase in their disorder, it has not been possible to accurately characterize the relationship of duration of ESES or duration of aphasia with language outcome. This is because language outcomes for this study were based on the last clinical observation for these patients, and it is still possible for these patients to show signs of relapse by their next follow-up appointment. Furthermore, due to non-uniformity in the timing of sleep EEGs, it can often be difficult to determine duration of ESES, accurately.

It is interesting to note, however, that the proportion of patients with ongoing ESES at the time of last observation, does seem to be higher in poorer language outcome groups (**Table 3-2**). Although this did not reach statistical significance, it suggests that the presence of ESES may potentially be associated with worse language outcome.

The next section examines the relationship between a history of a relapsing and remitting course and outcomes in adulthood, when clinical symptoms are expected to stabilize.

3.6 Factors Influencing Long Term Outcomes in Adulthood

Table 3-3 presents collected data variables in relation to long term adult outcome, measures (level of independence and language abilities). For most variables, there was no significant difference among the outcome groups. Data for individuals with ESES is limited by the fact that many of these individuals did not have sleep EEGs early in the course of their illness. There was no significant correlation between patients who had a relapsing-remitting course of illness and outcome in adulthood.

I did find that adult outcome appeared to be linked to non-verbal intelligence quotient (IQ). Below average non-verbal IQ at assessment during the course of illness may be predictive of worse outcome in adulthood. The difference among the different outcome groups in proportion of patients with identified motor difficulties during the course of their illness, also approached significance (p= 0.069). There was a larger proportion of patients with identified motor difficulties during the course of their illness in the dependent group for adult outcomes.

These observations IQ may be explained by the fact that for these individuals, cognitive difficulties and motor difficulties observed during the course of the illness persisted into adulthood, contributing to their ongoing dependence. It is possible that these individuals may represent a sub-group of LKS patients with a more severe overall developmental disorder.

Motor difficulties are not commonly reported in LKS, and may be neglected. These results suggest that it may be clinically important to assess patients with LKS for the presence of motor features, as these may have prognostic implications. In addition, early intervention with occupational therapy and physiotherapy may help some patients to achieve a better overall outcome.

Table 3-3: Differences in collected variables among adult outcome groups

	Independent with no/minimal language difficulties	Independent with significant language difficulties	Remains dependent	(*p* value)
No.:	14 (56%)	7 (28%)	4 (16%)	
Mean current age (range)	24y11m ± 4y0m (19y2m to 31y0m)	27y0m ± 5y5m (18y8m to 33y9m)	24y6m ± 5y0m (19y10m to 29y0m)	0.557*
Male%	57.1%	71.4%	75%	0.718†
Positive FH	42.9%	57.1%	50.0%	0.823†
Mean age at SLR (range)	5y3m ± 1y8m (1y6m to 7y11m)	5y6m ± 2y4m (1y7m to 8y10m)	4y0m ± 1y10m (2y to 5y10m)	0.465*
Prior speech and language delay	35.7%	42.9%	25%	0.838†
Clinical Sz	92.9%	85.7%	100.0%	0.692†
ESES on EEG (N.)‡	70.0% (7/10)	33.3% (1/3)	25% (1/4)	0.236†
< average NVIQ	14.3%	71.4%	100.0%	**0.002†**
BD	71.4%	85.7%	100.0%	0.409†
MD	21.4%	14.3%	75%	0.069†
Relapsing remitting course	50.0%	42.9%	75%	0.576†

BD: behavioural difficulties; MD: motor difficulties; ESES: electrical status epilepticus in slow wave sleep; FH= family history; No.= number of patients; NVIQ: non-verbal intelligence quotient; SLR: speech and language regression; Sz: seizure. *One-way Anova with Bonferroni correction; †Fisher's exact; ‡those without available sleep EEG reports (9/52, 17.3%) were excluded

3.7 Summary

This is the largest single cohort LKS study, with strict inclusion criteria for LKS patients. The follow-up data has advanced our understanding of LKS prognosis and long-term outcome. The cohort size has facilitated statistical analysis, identifying key factors that are likely to determine LKS outcome.

Through this clinical study, we can draw a number of important conclusions. Overall, evidence from this study supports the concept that LKS is a developmental and (possibly for some patients) neurogenetic disorder. Indeed, (i) the onset of speech and language regression is consistently at a particular developmental age with stabilization and plateauing in adolescence/adulthood and, (ii) a high proportion have a positive family history.

This study also found that early age of onset correlates with worse language outcomes. This is compatible with the "early vulnerability" theory and may be due to the fact that having an insult at an early crucial stage of neurodevelopment is particularly damaging because this may cause "flow on" effects to the development of subsequent neural connections and functional skills, outweighing the possible protective effects of neuroplasticity (Anderson et al., 2010). This finding highlights the importance of early intervention to limit damage to the developing brain.

My study confirms that behavioural and motor symptoms are significant co-morbidities. Below average non-verbal IQ and the presence of motor symptoms are associated with worse outcome in adulthood. This may be because these symptoms persist into adulthood, leading to dependence. It is also possible that these individuals have a more severe disruption of normal developmental processes.

Lastly, the study also highlights that approximately half of patients with LKS have relatively good outcomes in adulthood, achieving independence and either little or no speech difficulties. This observation has been consistent across many different cohort studies over the last 6 decades.

4 Chapter 4: Genotypic and Phenotypic Spectrum of *GRIN2A*- Landau Kleffner Syndrome

4.1 *GRIN2A*: The N2A Subunit of the NMDA receptor

The gene, *GRIN2A* (Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2A), is located on chromosome 16p13.2 and encodes the N2A subunit of the NMDA receptor. *GRIN2A* is highly expressed within the cerebral cortex, hippocampus cerebellum and striatum **(Figure 4-1).**

As described in Chapter 1.2.1, the NMDA receptor is a hetero-tetrameric ligand and voltage-gated channel. It comprises 2 obligatory glycine-binding subunits and 2 glutamate binding sub-units, which may be any combination of N2 (N2A, N2B, N2C or N2D) or N3 subunits (N3A or N3B).

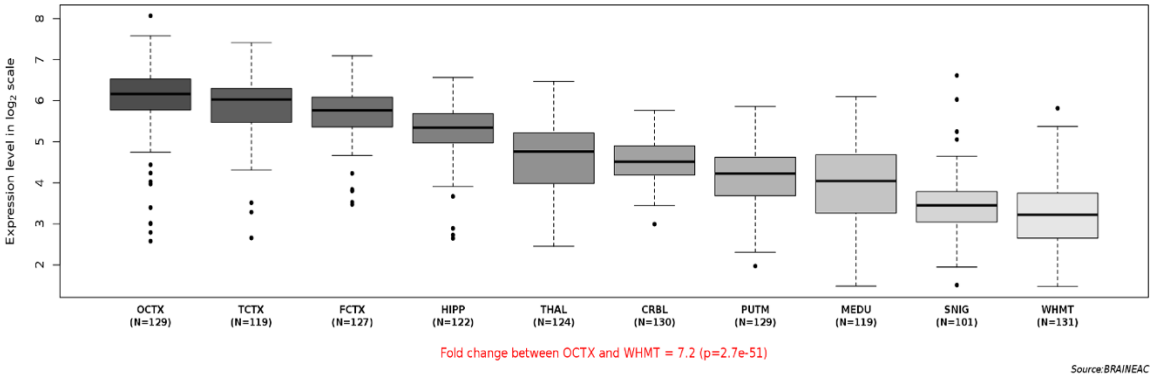
Each type of NMDA receptor subunit has unique electrophysiological and pharmacological properties. (Paoletti and Neyton, 2007). For instance, N2B rich NMDA receptors generate low amplitude slow currents that last twice as long as N2A mediated currents. This is thought to facilitate a large influx of Ca^{2+} ions promoting gene transcription and synapse potentiation in the early stages of brain development. In contrast, N2A rich NMDA receptors generate high amplitude, fast currents for transient synaptic communication in response to sensory stimuli (Bagasrawala et al., 2017).

NMDA receptor sub-units have specific spatio-temporal expression patterns. Studies in rodents and humans have shown that NMDA receptor expression within the developing human brain is evolutionarily conserved. N1 subunits are expressed in virtually all neurons throughout development. N2(A-D) and N3(A-B) have specific spatio-temporal patterns at different developmental stages. In the human fetal cerebral cortex, N1, N2A and N2B are expressed from the 16^{th} week of gestation. With increasing fetal development, the expression of N1 and N2A subunits increases, while the expression of N2B decreases. This developmental shift begins at approximately 24 weeks, and is thought to continue postnatally (Bagasrawala et al., 2017).

All NMDA subunits have 4 analogous domains as follows: (i) an extracellular N-terminal domain (NTD) with binding sites for sub- type specific allosteric modulators (e.g. Zn^{2+} for N2A), (ii) an extracellular ligand-binding domain for agonists, (iii) a channel pore-forming transmembrane domain with 4 hydrophobic segments (M1 to M4) with M2 only

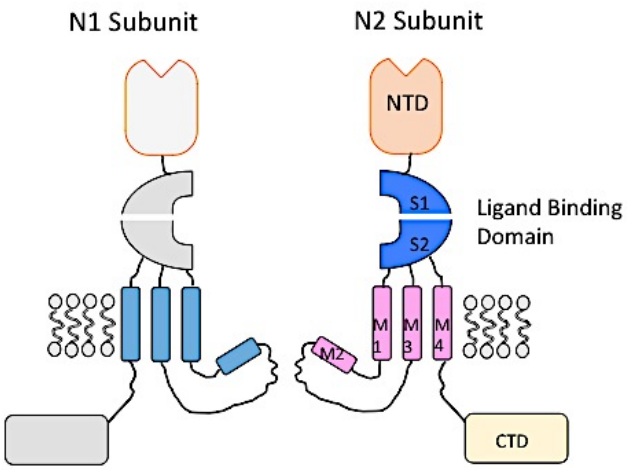
partially within the membrane, and (iv) an intracellular carboxyl-terminal domain for intracellular signalling **(Figure 4-2).**

Figure 4-1 Box plots for GRIN2A mRNA expression levels in adult brain regions (source: BRAINEAC; http://www.braineac.org/).



The expression levels are based on previously described exon array experiments and are plotted on a log2scale. (Trabzuni et al., 2011). OCTX: Occipital cortex; TCTX: Temporal cortex; FCTX: Frontal cortex; HIPP: Hippocampus; CRBL:Cerebellum; THAL: Thalamus; PUTM: Putamen; MEDU: Medulla; SNIG: Substantia Nigra; WHMT: White matter. "N" indicates the number of brain samples analysed to generate results for each brain region

Figure 4-2 The structural domains of the NMDA receptor, adapted from (Strehlow, 2015b)



NTD: N-terminal domain; S1 and S2: subunits of Ligand binding domain; M1-M4: transmembrane domains; CTD: carboxyl-terminal domain

4.2 Identification of *GRIN2A* mutations in Epilepsy Aphasia Spectrum

GRIN2A was first implicated in epilepsy aphasia spectrum disorders (EASD) in 2010., when heterozygous microdeletions of chromosome 16 (encompassing only this gene) were identified in 3 patients with epilepsy of varying severity, associated with centrotemporal spikes on EEG (Reutlinger et al., 2010). In addition, a separate study analysing copy number variants (CNVs) in a cohort of 61 patients with ECSWS and LKS reported a 109kb deletion of chromosome 16 encompassing *GRIN2A* (Lesca et al., 2012) **(Table 4-1).** Subsequently in 2013, 3 papers were published concurrently, describing *GRIN2A* variants in 8-20% of individuals with EASD (Lesca et al., 2013, Carvill et al., 2013b, Lemke et al., 2013). Indeed, the NMDA receptor's role in excitatory neurotransmission and long-term potentiation, renders *GRIN2A* an attractive candidate gene for these disorders.

To date, 51 different *GRIN2A* variants have been reported in EASD (**Table 4-2**). Some of these are recurrent variants, reported in more than one family. The majority of reported variants (36/51; 70.5%) are missense mutations. The remainder are stop mutations (7/51; 13.7%), splice-site variants (3/51; 5.9%), intragenic deletions or duplications leading to frame-shifts (4/51; 7.8%); and an in-frame deletion (1.9%).

The missense *GRIN2A* mutations reported in EASD are scattered across the different protein domains, with a greater concentration of variants around the S1 and S2 ligand binding domains (**Figure 4-3**). Only 2 missense variants have been reported in the transmembrane linker regions.

Only 6 (18.2%) of the mutations reported to date occurred *de novo*. Of these, 3 have also been reported with dominant inheritance in different families. All the reported *de novo* mutations in EASD are either within the key S1 and S2 ligand binding domains or their adjacent linker regions, suggesting that mutations within these domains are less likely to be tolerated **(Figure 4-3).**

Approximately half the GRIN2A mutations reported in EAS are familial variants (49%). 63% of reported familial variants are associated with incomplete penetrance, with at

least one non-disease manifesting carrier within the family (**Table 4-2**). Even when a variant is reported to segregate with disease status within a family, there is often marked intra-familial phenotypic variability. Related individuals carrying the same *GRIN2A* variant may have variable cognitive abilities (from global learning difficulties to solely speech and language difficulties) and different forms of childhood epilepsy (CECTS, LKS, ECSWS) **(Section 4.3).**

Table 4-1: Reported GRIN2A Copy Number Variants in Epilepsy Aphasia Spectrum Disorders

CNV coordinates GrCH37/hg19	Genes within the CNV	Phenotype	Inheritance (Segregates)*	Reference
16:8,900,000-9,900,000	*PMM2, USP7, C16orf72, LINC02177, LINC01177, GRIN2A*	CECTS	De novo	(Reutlinger et al., 2010)
arr16p13.2; 16:9,915,756-9,915,815 (≤15kb)	*GRIN2A*	LKS	Inherited (Y)	(Lesca et al., 2013)
arr16p13.2; 16:10,246,239-10,321,593 (≤75kb)	*GRIN2A*	ECSWS and verbal dyspraxia	Inherited (N)	(Lesca et al., 2013)
arr16p13.2; 16: 10,246,239-10,354,862 (≤109kb)	*GRIN2A*	LKS	De novo	(Lesca et al., 2012)
arr16p13.2 16: 9,850,000-10,100,000†	*GRIN2A*	CECTS	Unknown	(Lemke et al., 2013)
arr16p13.2 16: 9,850,000-9,900,000†	*GRIN2A*	IEAD	Unknown	(Lemke et al., 2013)
arr16p13.2 16:10,250,000-10,300,000†	*GRIN2A*	CECTS	Unknown	(Lemke et al., 2013)

*Segregation: non-segregation is defined as having one or more asymptomatic mutation carrier(s) within the family

† Coordinates estimated from Supplementary Figure on original paper. CECTS: Childhood epilepsy with centrotemporal spikes; ECSWS: Epilepsy with continuous spike waves in slow wave sleep; IEAD: Intermediate epilepsy aphasia disorder; LKS: Landau Kleffner syndrome; N: No; Y: Yes.

Table 4-2: Reported GRIN2A Mutations within Epilepsy Aphasia Spectrum Disorders

Mutation	Phenotype	Inheritance/ (Segregates*)	dbSNP	1000G MAF	gnomAD A.F.	EVS	Provean	SIFT	Polyphen	Functional effect on protein
N-Terminal Domain										
c.2T>C p.Met1Thr[1]	LKS	Inherited (Y)	rs397518466	Absent	-Absent	Absent	-0.78	0.00	0.213	Alters translation start codon
c.236C>G p.Pro79Arg[2]	CECTS, ECSWS	Inherited (N)	Absent	Absent	0.00041%	Absent	-7.24	0.002	1.00	Decreased agonist potency[3] Reduced cell surface expression[3]
c.547T>A p.Phe183Ile[2]	CECTS	Inherited (N)	rs587780353	Absent	0.0040%	Absent	-5.44	0.00	1.00	Not done
c.551T>G p.Ile184Ser[4]	ECSWS	Inherited (N)	Absent	Absent	Absent	Absent	-4.40	0.002	0.999	Decreased agonist potency and reduced cell surface expression[5]
c.691T>C p.Cys231Arg[6]	IEAD	Unknown	Absent	Absent	Absent	Absent	-10.46	0.001	1.00	Not done
c.692G>A p.Cys231Tyr[2]	LKS	Inherited (N)	Absent	Absent	Absent	Absent	-9.70	0.00	1.00	Decreased agonist potency Reduced total protein expression Reduced cell surface expression[3]
c.728C>T p.Ala243Val[2]	CECTS and LD	Unknown	Absent	Absent	Absent	Absent	-3.73	0.001	1.00	Loss of high affinity inhibition by Zn^{2}
c.869C>T p.Ala290Val[2]	CECTS	Unknown	rs199528312	Absent	0.0033%	Absent	-2.21	0.094	0.593	Not done
c.883G>A p.Gly295Ser[4]	CECTS	Unknown	rs568484876	<0.01	0.0049%	Absent	-5.02	0.004	0.971	Not done
c.1108C>T p.Arg370Trp[2]	CECTS	Unknown	rs761168789	Absent	0.00041%	Absent	-7.69	0.001	1.00	Not done
S1 Ligand Binding Domain										
c.1306T>C p.Cys436Arg[2]	IEAD	De novo	Absent	Absent	Absent	Absent	-11.77	0.00	0.985	Increased agonist potency[7] Reduced agonist potency[3] Reduced cell surface expression[3]
c.1364G>A p.Cys455Tyr[6]	IEAD	Unknown	Absent	Absent	Absent	Absent	-10.68	0.001	1.00	Not done

Mutation	Phenotype	Inheritance/ (Segregates*)	dbSNP	1000G MAF	gnomAD A.F.	EVS	Provean	SIFT	Polyphen	Functional effect on protein
c.1447G>A p.Gly483Arg[4]	ECSWS	Inherited (N)	Absent	Absent	Absent	Absent	-7.63	0.001	1.00	Reduced agonist potency and reduced cell surface expression[3,7]
c.1510C>T p.Arg504Trp[4]	ECSWS	Inherited (N)	Absent	Absent	0.0035%	Absent	-6.60	0.00	1.00	Increased agonist potency[7]
c.1553G>A p.Arg518His[4,8]	LKS	Inherited (Y)/De novo	rs397518470	Absent	Absent	Absent	-4.82	0.00	1.00	Prolongs channel opening time[4,5,7] Decreased agonist potency Reduced surface expression
c.1592C>T pThr531Met[1]	ECSWS	Inherited (Y)	Absent	Absent	Absent	Absent	-6.60	0.00	1.00	Prolongs channel opening[1] Reduced agonist potency[7]
Linker regions and M1 to M4 Transmembrane domains										
c.1642G>A p.Ala548Thr[4]	LKS	De novo	Absent	Absent	Absent	Absent	-3.86	0.001	0.924	Not done
c.1954T>G p.Phe652Val[4]	ECSWS	De novo	rs397518471	Absent	Absent	Absent	-6.55	0.001	0.999	Prolongs channel opening time[4]
c.2441T>C p.Ile814Thr[2]	CECTS	Inherited (N)	rs780654733	Absent	0.0014%	Absent	-3.90	0.029	0.157	No functional deficit[3]
S2 Ligand Binding Domain										
c.2007G>T p.Lys669Asn[4]	ECSWS	Unknown	Absent	Absent	Absent	Absent	-4.74	0.001	1.00	Increased agonist potency[7]
c.2081T>C p.Ile694Thr[4]	LKS	De novo	Absent	Absent	Absent	Absent	-4.79	0.00	1.00	Decreased agonist potency[7]
c.2095C>T p.Pro699Ser[2]	CECTS	De novo	Absent	Absent	Absent	Absent	-4.04	0.104	1.00	Increased agonist potency[7]
c.2113A>G p.Met705Val[2]	CECTS	Inherited (N)	Absent	Absent	Absent	Absent	-3.81	0.00	0.996	Decreased agonist potency Decreased total and cell surface expression[3,7]
c.2140G>A p.Glu714Lys[2]	ECSWS	Unknown	Absent	Absent	Absent	Absent	-2.50	0.148	0.190	No significant effect on agonist potency[7] Reduced total and surface[3] expression

Mutation	Phenotype	Inheritance/ (Segregates*)	dbSNP	1000G MAF	gnomAD A.F.	EVS	Provean	SIFT	Polyphen	Functional effect on protein
c.2146G>A p.Ala716Thr[4,9]	IEAD with verbal dyspraxia/ LKS	Inherited (Y)/De novo	rs762659685	Absent	0.00041%	Absent	-3.86	0.001	1.00	Reduced agonist potency[7] No significant effect[5]
c.2179G>A p.Ala727Thr[2]	CECTS	Unknown	Absent	Absent	Absent	Absent	-3.57	0.001	0.993	Reduced agonist potency[7]
c.2191G>A p.Asp731Asn[4,10]	IEAD/LKS	Inherited (Y)/De novo	rs796052549	Absent	Absent	Absent	-4.46	0.001	1.00	Reduced agonist potency Reduced current amplitude Decreases channel open probability Increased sensitivity to negative allosteric modulators Reduced total and cell surface expression[3,7,11]
c.2200G>C p.Val734Leu[2]	CECTS	Inherited (N)	Absent	Absent	Absent	Absent	-2.63	0.001	0.995	Reduced agonist potency[7]
c.2278G>A p.Gly760Ser[6]	LKS	De novo	Absent	Absent	Absent	Absent	-5.38	0.009	1.00	Not done
c.2314A>G p.Lys772Glu[2]	IEAD	Unknown	Absent	Absent	Absent	Absent	-3.48	0.009	0.996	Reduced agonist potency[7]
C- Terminus Domain										
c.2710A>T p.Ile904Phe[2]	CECTS	Inherited (Y)	Absent	Absent	Absent	Absent	-1.41	0.013	0.411	Not done
c.2797G>A p.Asp933Asn[4]	LKS	Inherited (N)	rs933322445	Absent	Absent	Absent	-2.33	0.010	0.997	No functional deficit[3]
c.2927A>G p.Asn976Ser[2]	ECSWS	Unknown	rs886039239	Absent	0.00081%	Absent	0.089	-1.28	0.186	No functional deficit[3]
c.3751G>A p.Asp1251Asn[4]	IEAD	Inherited (Y)	Absent	Absent	Absent	Absent	-2.42	0.00	0.999	Not done

Mutation	Phenotype	Inheritance/ (Segregates*)	dbSNP	1000G MAF	gnomAD A.F.	EVS	Provean	SIFT	Polyphen	Functional effect on protein
c.3827C>G p.Ala1276Gly[4]	ECSWS	Unknown	rs145063086	<0.01	0.057% 1 HZ	EA: C=0.10% AA: C=0.02%	-0.78	0.284	0.517	Not done
c.4153G>T p.Asp1385Tyr[6]	IEAD	Inherited (N)	Absent	Absent	Absent	Absent	-0.75	0.023	1.00	Not done
Protein Truncating Variants										
c.90delTins(T)2 p.Pro31Serfs*107[2]	CECTS	Inherited (N)*	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.1585delG p.Val529Trpfs*22[2]	CECTS	Inherited (Y)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.1586delT p.Val529Trpfs*22[12]	ECSWS	Unknown	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.2334_2338delCTTGC p.Leu779Serfs*5[2]	IEAD/ ECSWS	Inherited (N)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.594G>A p.Trp198*[2]	IEAD	Unknown	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.1001T>A p.Leu334*[2]	ECSWS	Unknown	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.1818G>A p.Trp606*[12]	IEAD	Unknown	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.2041C>T p.Arg681*[2]	LKS	Inherited (N)	rs397518472	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.2407G>T p.Glu803*[12]	LKS	De novo	rs397518472	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.2829C>G p.Tyr943*[2]	CECTS/ ECSWS	Inherited (Y)	rs397518467	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done
c.4161C>A p.Tyr1387*[4]	ECSWS	Inherited (Y)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done

Mutation	Phenotype	Inheritance/ (Segregates*)	dbSNP	1000G MAF	gnomAD A.F.	EVS	Provean	SIFT	Polyphen	Functional effect on protein
c.1007+1G>A[1,2,4]	LKS, IEAD, ECSWS	Inherited (N)	rs397518465	Absent	Absent	Absent	N.A.	N.A.	N.A.	Disruption of donor splice site and nonsense mediated decay of mutant transcript
c.1123-2A>G[4]	LKS	Inherited (N)	rs397518469	Absent	Absent	Absent	N.A.	N.A.	N.A.	Skipping of exon 5 confirmed by RT-PCR and cDNA sequencing
c.2007+1G>A[2,12]	ECSWS	Unknown/ Inherited (N)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Predicted to affect splicing
c.1637_1639delCTT p.Ser547del[2]	IEAD/ ECSWS	Unknown	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.	Not done

AF: Allele frequency, CECTS: Benign epilepsy with centrotemporal spikes; ECSWS: Epilepsy with continuous spike waves in slow wave sleep; EVS: exome variant server, HZ: homozygous, IEAD: Intermediate epilepsy aphasia disorder; LKS: Landau Kleffner syndrome; MAF: minor allele frequency, N: no; N.A.:not applicable; Y: yes

*Segregation: non-segregation is defined as having one or more asymptomatic mutation carrier(s) within the family

[1](Carvill et al., 2013b); [2](Lemke et al., 2013); [3](Addis et al., 2017); [4](Lesca et al., 2013); [5](Sibarov et al., 2017); [6](Yang et al., 2017); [7](Swanger et al., 2016); [8](Conroy et al., 2014); [9](Fainberg et al., 2016); [10](Dyment et al., 2015); [11] (Gao et al., 2017); [12](von Stulpnagel et al., 2017)

Figure 4-3: Reported GRIN2A Mutations within Epilepsy Aphasia Spectrum Disorders

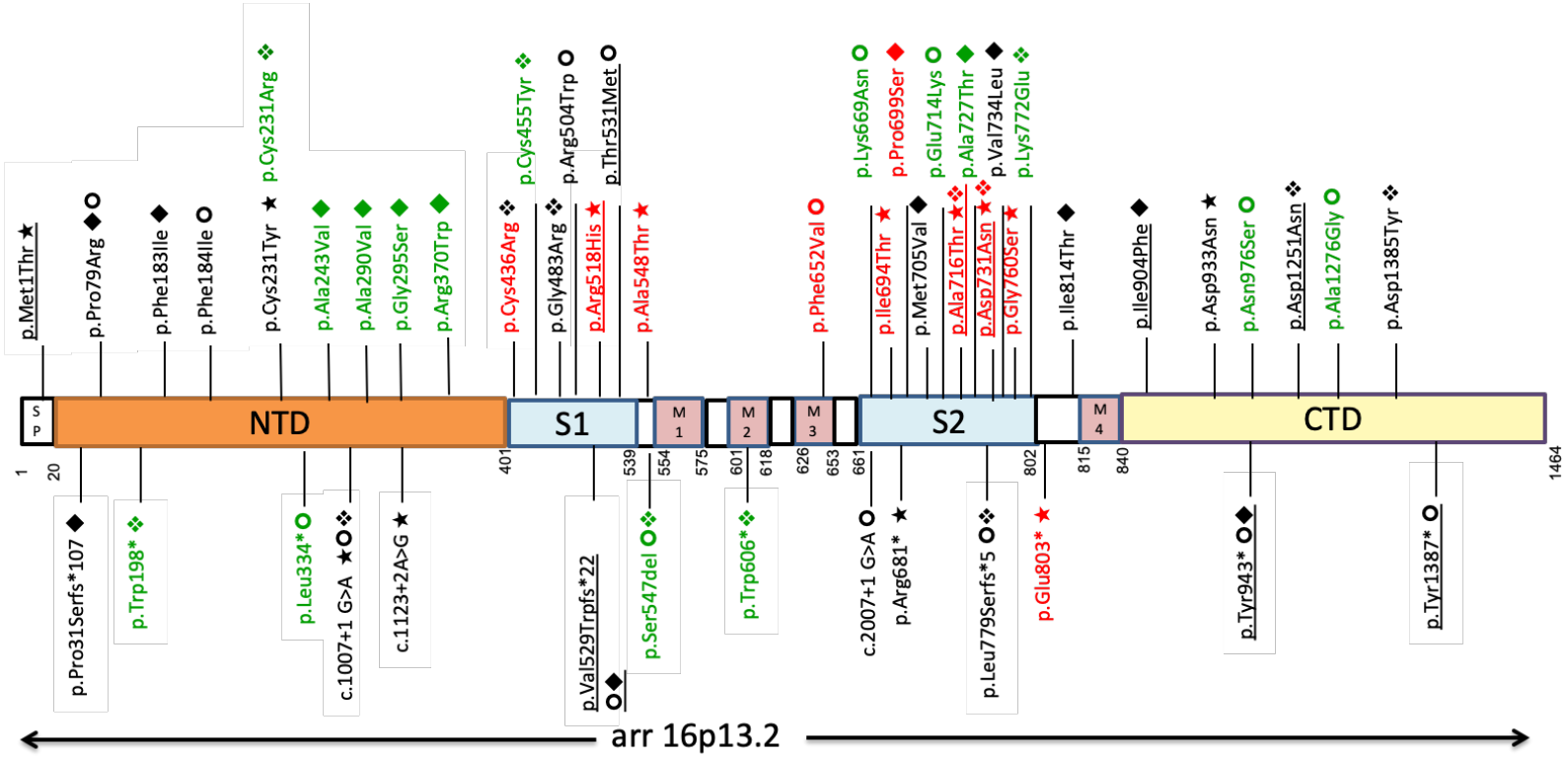


Figure Legend: Variants in red = *de novo* variants. Variants in black= Familial dominantly inherited variants. Variants in green = unknown inheritance. Underlined inherited variants= segregates within the family (no carriers without manifest disease), underlined de novo mutations: this variant has also been reported as a familial variant with good segregation.

★ Landau Kleffner Syndrome (LKS), ◆ Benign epilepsy with centrotemporal spikes (CECTS), O Epilepsy with continuous spike waves in slow wave sleep (ECSWS), ❖ Intermediate epilepsy aphasia disorder (IEAD)

4.3 Reported *GRIN2A* mutations: incomplete penetrance, variable expressivity and phenotypic pleiotropy

In addition to EASD, *GRIN2A* mutations have also been reported in other epileptic encephalopathies, and neurodevelopmental disorders such as schizophrenia, autism, dystonia and movement disorders (Tarabeux et al., 2011, Fernandez-Marmiesse et al., 2018). Within EASD, *GRIN2A* mutations often show incomplete penetrance and variable expressivity within families.

Incomplete penetrance, variable expressivity and phenotypic pleiotropy are not novel phenomena in genetic forms of childhood epilepsy. Incomplete penetrance (where a non-disease manifesting, asymptomatic parent, sibling or other relative harbours the disease-causing mutation) is well documented in *KCNT1* related epilepsy of infancy with migrating partial seizures (McTague et al., 2018) and autosomal dominant focal epilepsy associated with *CHRNA4* and *LGI1* (Rosanoff and Ottman, 2008, Leniger et al., 2003). In the 10% of *SCN1A*-related Dravet Syndrome associated with familial mutations, there is often variable expressivity; affected family members may present with milder phenotypes such as febrile seizures or generalized epilepsy with febrile seizures (GEFS+) syndromes (Depienne et al., 2010). In addition, phenotypic pleiotropy is well-recognized for several childhood epilepsy genes including *KCNQ2, ARX and SCN8A* (McTague et al., 2016).

The underlying basis for incomplete penetrance, variable expressivity and phenotypic differences remain yet to be fully elucidated, but may be attributed to: (i) mosaicism, (ii) type and location of mutation, (iii) the effect of other genes, (iv) epigenetics and (v) environmental influence.

4.3.1 Mosaicism

Mosaicism refers to the existence of more than one population of cells with different genotypes within an organism. Somatic mosaicism occurs post-conception during embryonic development and results in cells of different tissue types being affected to different degrees by a given gene mutation. This may partly explain why some

pathogenic mutations can be inherited from unaffected or mildly affected parents, and is well documented for Dravet Syndrome associated with *SCN1A* mutations (Depienne et al., 2010). While this has not been reported in the literature for *GRIN2A* and EASD, it is certainly theoretically possible that somatic mosaicism (and variable brain expression of mutant N2A) may also account for the incomplete penetrance and variable expressivity observed in these disorders.

4.3.2 Type and Location of Mutation

The type and location of a gene variant can determine the functional effect of the mutation on its encoded protein, thereby leading to phenotypic pleiotropy. For example, in *CDKL5*-associated developmental epilepsy, patients with missense mutations in the ATP binding site of the protein tend to have less severe phenotypes than those with mutations in the kinase domain (McTague et al., 2016). *SCN8A* gain-of-function missense mutations are associated with epileptic encephalopathy, whereas loss-of-function truncating *SCN8A* mutations are associated with intellectual disability without seizures (Blanchard et al., 2015). Furthermore, whilst heterozygous protein-truncating *SMC1A* mutations lead to a severe early onset epileptic encephalopathy, missense and small in-frame deletions lead to an almost entirely different phenotype – Cornelia de Lange Syndrome (Symonds et al., 2017).

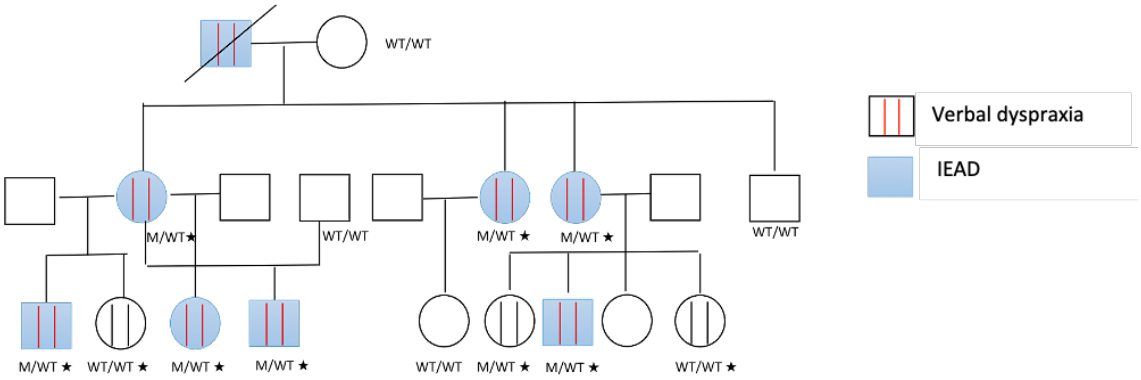
Functional investigations have now been undertaken on a wide range of reported *GRIN2A* mutations encompassing several different phenotypes including all EASD, other epileptic encephalopathies, intellectual disability, schizophrenia and movement disorders (**Table 4-2**) (Addis et al., 2017, Sibarov et al., 2017, Gao et al., 2017, Yuan et al., 2014, Swanger et al., 2016, Fernandez-Marmiesse et al., 2018). This is discussed further in **Section 4.6**. Most reported missense *GRIN2A* mutations within EASD occur outside the linker-transmembrane domains and have been found to have an overall loss-of-function effect. Beyond EASD, when all reported phenotypes associated with *GRIN2A* are taken into consideration (including other epileptic encephalopathies), it has emerged that missense mutations within linker transmembrane domains tend to have overall gain of function effects and are associated with more severe phenotypes (Strehlow et al., 2019).

4.3.3 Effect of other genes

It is likely that an individual's genetic background can influence the phenotypic manifestation of any given gene mutation in childhood epilepsy. For example, studies performed in the *SCN1A* mouse model suggest that concurrent *SCN8A and SCN9A* mutations can respectively raise or lower seizure thresholds in *SCN1A* related epilepsy (Meisler et al., 2010).

With the advent of widely available next generation sequencing technologies, it is also now increasingly recognized that patients with epilepsy may have more than one potentially "causative" gene mutation for their clinical phenotype. In these cases, it is possible that these mutations in different epilepsy genes may have synergistic effects. For example, a child with neonatal seizures with ictal vocalization evolving to West Syndrome was recently reported to have both a *de novo* mutation in *SCN2A* and a *de novo* gain-of-function *GRIN2A* mutation (p.Val452Met), previously reported in a patient with schizophrenia (Singh et al., 2016). In addition, affected individuals in a family with IEAD and verbal dyspraxia were reported to have both a loss-of-function p.Ala716Thr *GRIN2A* mutation and the p.Asn327Ser variant in *SRPX2,* a gene implicated in the formation of the perisylvian cortex (Lesca et al., 2013). In this kindred, the *SRPX2* mutation was evident in all clinically affected family members. The *GRIN2A* variant showed a more incomplete pattern of penetrance, evident in 7/7 family members with seizures, 1 family member without seizures, and 8/10 family members with verbal dyspraxia **(Figure 4-4).**

Figure 4-4 Family tree showing segregation of *GRIN2A* p.Ala716Thr mutation and *SRPX2* p.Asn327Ser mutation in a family described by (Lesca et al., 2013)



M/WT: heterozygous for *GRIN2A* mutation; WT/WT: *GRIN2A* negative, ★: *SPRX2* positive

4.3.4 Epigenetics

The concept of "epigenetics" refers to alterations of the chromatin template that bring about varying patterns of gene expression in response to external (environmental) or internal (developmental) stimuli, without changes in DNA sequence (Kobow and Blumcke, 2018). Recent research has shown that epigenetic mechanisms including DNA methylation, histone modification, chromatin remodelling and non-coding RNA exert important influence on gene networks in epilepsy (Hauser et al., 2018). As such, it is extremely likely that these mechanisms contribute to incomplete penetrance, variable expressivity and phenotypic pleiotropy in genetic epilepsy.

Conroy et al (2014) performed genome wide methylation analysis in 2 discordant monozygotic twin pairs with *GRIN2A*- negative LKS, and found fourteen loci (13 genes) with significant differential methylation patterns in both twin sets. However, when the methylation profiles of affected individuals were compared with those of normal controls, there were no significantly different loci (Conroy et al., 2014).

4.3.5 Environment

The interplay between genes and environment can influence epilepsy phenotype. A study of concordance rates among 11,900 Danish twin pairs estimated that individual specific environmental factors accounted for approximately 30% of susceptibility to epilepsy development in younger individuals (Kjeldsen et al., 2001).

Minor head injury has been reported to trigger seizures in individuals carrying *CACNA1A* mutations (Stam et al., 2009). The course of many genetic neurometabolic/mitochondrial disorders is also often altered by environmental factors such as infection (Saudubray and Garcia-Cazorla, 2018).

Whilst no study has formally examined the influence of environmental factors in LKS or EASD, there have been reports of symptoms of LKS occurring after minor head injury or infection (Bhardwaj et al., 2009). These include 4 of the 6 patients described in William Landau's and Frank Kleffner's original report (Landau and Kleffner, 1957)**(Chapter 1; Table 1-2**). Some of the patients within our GOSH cohort of LKS patients have also

reported similar events preceding symptom onset, including one child with a *GRIN2A* mutation (see clinical summaries in **Appendix**).

4.4 *GRIN2A* Analysis of GOSH LKS cohort

Sanger sequencing and multiplex ligation probe amplification (MLPA) were carried out to establish the frequency of *GRIN2A* mutations within the GOSH LKS cohort.

The methods used for Sanger sequencing and MLPA were described in detail in **Chapter 2; Sections 2.3.2 and 2.3.3**. For *GRIN2A* primer design, the DNA template was taken from Ensembl genome browser (http://www.ensembl.org/index.html), NCBI Genome Reference Consortium (GRC)h38.p7; chromosome 16: 9,753,404 – 10,182,754; NM_000833.4. Primer pairs for exon-specific PCR amplification of genomic exons and flanking intron boundaries were designed using Primer3 software (http://bioinfo.ut.ee/primer3/), based on all Ensembl coding transcript variants. Primer sequences and annealing temperatures are presented in **Table 4-3**.

Of the 91 individuals on the GOSH database of patients who consented to participate in this study, DNA was available for 62 families. Prior to referral, 7 patients had already had targeted sequencing for variants in *GRIN2A* in diagnostic laboratories through single gene testing, or epilepsy gene panels. *GRIN2A* mutations were identified in 2/7 of these patients. I performed Sanger sequencing on the remaining 55 patients with available DNA, and identified 4 other patients with *GRIN2A* sequence variants (**Figure 4-5).**

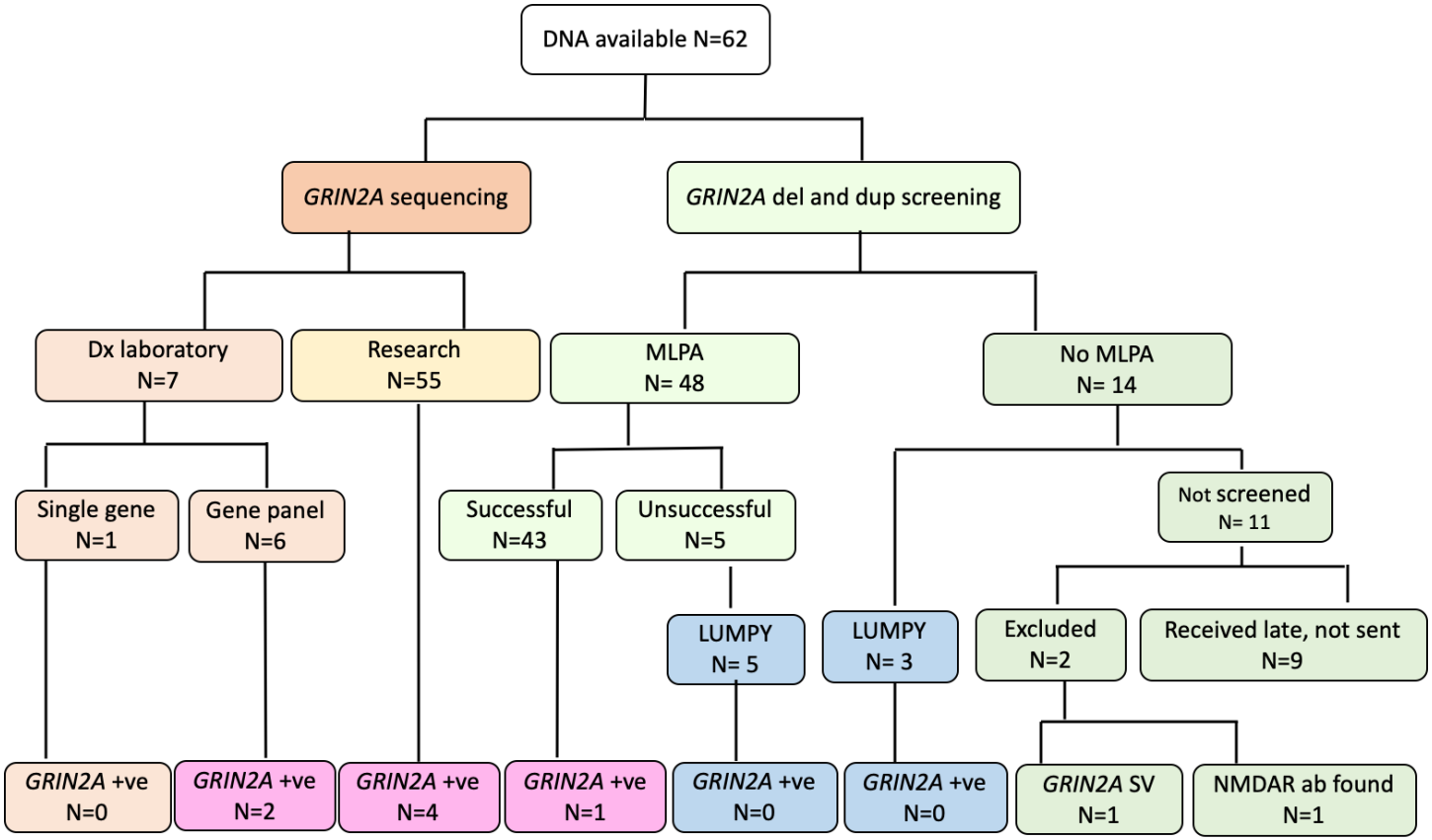
I also worked with the Great Ormond Street Hospital (GOSH) for Children/ North East Thames Regional Genetics Service, to perform *GRIN2A* multiplex ligation probe amplification (MLPA) analysis for 48 patients. This identified one patient with a *GRIN2A* deletion, also found on concurrent diagnostic microarray. For 5 patients, the MLPA run did not work. These patients eventually had whole genome sequencing and had copy number variant (CNV) screening by GOSgene bioinformaticians (led by Hywel Williams) using LUMPY (Layer et al., 2014) (**Chapter 2, Section 2.3.5)**.

DNA for 14 patients arrived after the MLPA run was completed. 3 of these were sent for whole genome sequencing and had CNV screening by GOSgene bioinformaticians using LUMPY. 2 of these individuals were excluded from further genetic screening – 1

was identified to have a *GRIN2A* mutation on epilepsy gene panel performed by the Great Ormond Street Hospital Diagnostic laboratory; the other was later identified to have N-methyl-D-aspartate (NMDA) receptor antibodies. The remaining 9 had whole exome sequencing and could not have CNV screening via LUMPY. Due to budget constraints, these individuals have yet to be screened for *GRIN2A* CNVs (**Figure 4-5**).

Whilst genetic analysis was ongoing for this cohort, phenotypic data collection was completed. After review of phenotypic data, I determined that 17 patients did not have classical LKS (**Chapter 3, Figure 3-1**). As such, a total of 7 *GRIN2A* mutations were identified in 45 patients (15.5%) with classical LKS within the GOSH cohort; of these, 4 are novel, unpublished mutations. 3 of the *GRIN2A* mutations we identified occurred *de novo*; 2 were inherited from unaffected parents. 1 mutation was inherited from a mother who had a history of childhood epilepsy and speech and language difficulties. This proband's younger brother also carries this mutation and has also been diagnosed with LKS. For 1 proband, no parental DNA was available **(Table 4-4; Figure 4-6 and Figure 4-7).** The clinical presentations of all these patients have been summarised in the **Appendix**.

Figure 4-5: Screening the LKS Cohort for *GRIN2A* sequence variants, deletions and duplications



DNA: deoxyribonucleic acid; del: deletion; dup: duplication; Dx: diagnostic; MLPA: multiplex ligation probe amplification; N: number of patients; NMDAR ab: N-methyl-D-aspartate receptor antibodies; SV: sequence variant

Table 4-3: Primer Sequences used for GRIN2A Sequencing

Transcript Number								Primer Sequence	Annealing Temperature (°C)	PCR Product Size (base pairs, bp)
001	**002**	**006**	**201**	**202**	**004**	**012**	**013**			
3.1F	2.1F	2.1F				2.1F	2.1F	ATCCTGCTGCTGAGTTCCAT	60	348bp
3.1R	2.1R	2.1R				2.1R	2.1R	GCAGAGCTACCACGTTCAC		
3.2F	2.2F	2.2F				2.2F	2.2F	GGTGATGCTGGGTCACAG	60	379bp
3.2R	2.2R	2.2R				2.2R	2.2R	TCCGAAGACCTGCAGCAG		
4.1F	3.1F	3.1F	1.1F	1.1F	2.1F			ACTTTCCTTACTTCTAGGACGCA	60	467bp
4.1R	3.1R	3.1R	1.1R	1.1R	2.1R			GTGTTCCCAGAGACCAAGCT		
4.2F	3.2F	3.2F	1.2F	1.2F	2.2F			TCCAAAGACGAGGCTGTTCT	60	378bp
4.2R	3.2R	3.2R	1.2R	1.2R	2.2R			CAAGCAAACAATGACAACAGCA		
5F	4F	4F	2F	2F				TGGCATCCACTTATTTGTTCGAT	60	250bp
5R	4R	4R	2R	2R				GAGCCCCTTTATTGCCCATG		
6F	5F	5F	3F	3F				TGCCTCTCCAGAAATCAGCG	60	311bp
6R	5R	5R	3R	3R				CAAAGGGTTGGGCACGTTC		
7F	6F	6F	4F	4F				GGAAAGCCACTTCCCCTTTT	60	366bp
7R	6R	6R	4R	4R				TCCTGCTAACATTCCTGAGGA		
8F	7F	7F	5F	5F				TACTCAGACAAAGGGCCTGC	60	343bp
8R	7R	7R	5R	5R				CCTTTCTGGTCCCATCCTCT		
9F	8F	8F	6F	6F				GCAGCAAGTCGAAATTGTGC	60	393bp
9R	8R	8R	6R	6R				TGCCTGGTCTAGAGTAATGTGT		
10F	9F	9F	7F	7F				CTGCGTGGTTGTCATACACA	60	390bp

Transcript Number								Primer Sequence	Annealing Temperature (^{O}C)	PCR Product Size (base pairs, bp)
001	**002**	**006**	**201**	**202**	**004**	**012**	**013**			
10R	9R	9R	7R	7R				TCAATGAGAGGCACCTGAATCT		
11F	10F	10F	8F	8F				TGCATTTACCTCCTAACACCAG	60	330bp
11R	10R	10R	8R	8R				AAGGTTTATCGCTCGCAGAC		
12F	11F	11F	9F	9F				AGTGTGGGATGCTTTCAGGA	60	355bp
12R	11R	11R	9R	9R				GAAGCCCAGGAGCAAACAAA		
13F	12F	12F	10F	10F				TCGTCTGTTCCAAACCCAGA	58	389bp
13R	12R	12R	10R	10R				CATCAAGAACCCAAGCGCTT		
14.1F	13.1F	13.1F	11.1F	11-12.1F				GGTTTCATGTTCACTGCTGC	60	446bp
14.1R	13.1R	13.1R	11.1R	11-12.1R				CCGTGTTAGGGTTGGACTCA		
14.2F	13.2F	13.2F	11.2F	11-12.2F				GGAGACAACATGAACGAACTCC	60	474bp
14.2R	13.2R	13.2R	11.2R	11-12.2R				TCTAGGGGAGCTTGATTTGGT		
14.3F	13.3F	13.3F	11.3F	11-12.3F				AGAACCACAAAACCAAGGACA	60	484bp
14.3R	13.3R	13.3R	11.3R	11-12.3R				CAGGCATCGCACTTGAAGG		
14.4F	13.4F	13.4F	11.4F	11-12.4F				TTCCCCGCACAGTGAGAC	60	478bp
14.4R	13.4R	13.4R	11.4R	11-12.4R				AGACTTGCTCCTCTTGCTGT		
14.5F	13.5F	13.5F	11.5F	11-12.5F				GTCGACAAACCTAGGGAGCT	60	600bp
14.5R	13.5R	13.5R	11.5R	11-12.5R				GCAGGGCACTATTGGACATC		

Table 4-4: *GRIN2A* mutations within the GOSH LKS Cohort

Case	Mutation	Reported/Novel	Means of identification	Inheritance/ (Segregates)	dbSNP	1000G MAF	GnomAD A.F.	EVS	Provean	SIFT	Polyphen
9	c.2041C>T p.Arg681*	Reported[2]	Research Sanger sequencing	NPS	rs397518472	Absent	Absent	Absent	N.A.	N.A.	N.A.
11	c.1552C>T p.Arg518Cys	Novel	Research Sanger sequencing	UF (No)	rs747838255	Absent	0.00041%	Absent	-7.71	0.00	1.00
49	c.1289_1290delGT p.Val430Glufs*18	Novel	Research Sanger sequencing	UM (No)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.
67	c.1776_1777dupAA p.Ala593Lysfs*62	Novel	Research Sanger sequencing	*de novo*	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.
68	arr 16p13.2p13.13 (9,287840-10,889,600) x1	Reported[3]	MLPA and diagnostic microarray	*de novo*	rs397518469	Absent	Absent	Absent	N.A.	N.A.	N.A.
79	c.1553G>A p.Arg518His	Reported[1]	Diagnostic gene panel	*de novo*	rs397518470	Absent	Absent	Absent	-4.82	0.00	1.00.
95	c.3212_3221del p.His1071Leufs*33	Novel	Diagnostic gene panel	AM (Yes)	Absent	Absent	Absent	Absent	N.A.	N.A.	N.A.

AF: Allele frequency; AM: affected mother; MAF: minor allele frequency, N.A.: not applicable; NPS: no parental samples; UF: Inherited from unaffected father; UM: Inherited from unaffected mother. [1](Lesca et al., 2013); [2](Lemke et al., 2013); [3](Reutlinger et al., 2010)

Figure 4-6: *GRIN2A* Sequence Chromatograms

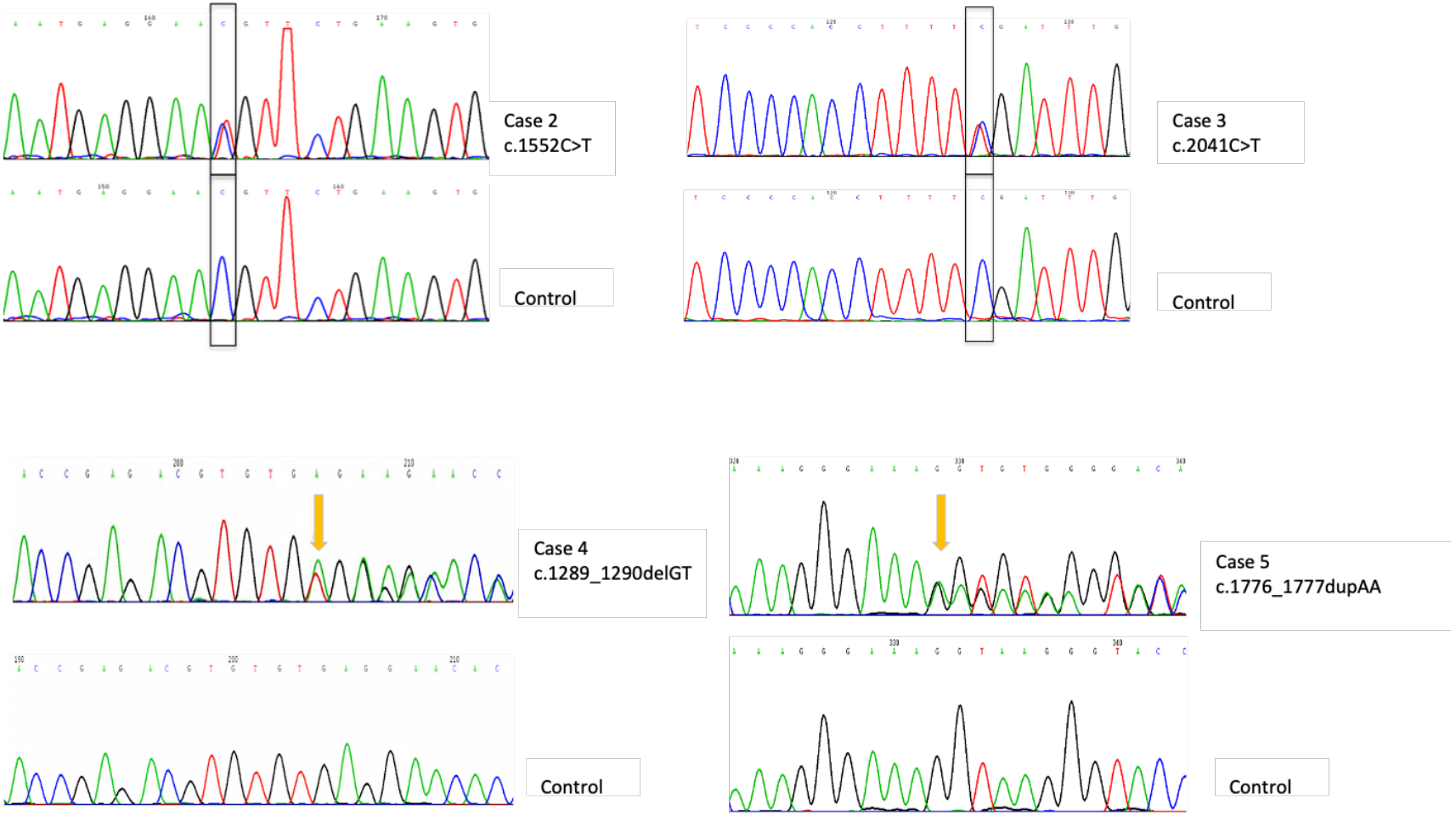
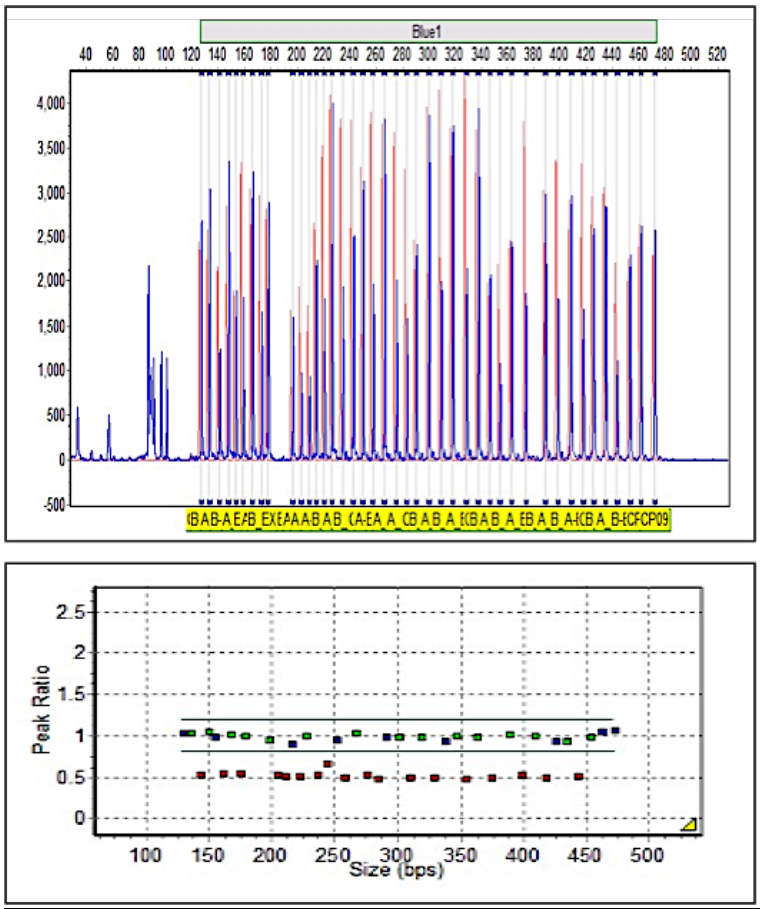


Figure Legend: Colours represent different nucleotide bases – green: adenine, red: thymine, black: guanine, blue: cytosine. Rectangles indicate position of missense variants. Arrows indicate position of deletions or duplications leading to frameshifts

Figure 4-7: MLPA Result for Patient 7 showing deletion of entire *GRIN2A* gene



	Probe Nam	Bin Size	5_-_14G03180_A
1	A-EX05	141.4	0.571
2	A-EX06	209.8	0.539
3	A-EX09	418.2	0.508
4	A-EX10	203.5	0.502
5	A-EX12	257.7	0.507
6	A-EX14B	220.9	0.512
7	A_EX01	374.5	0.491
8	A_EX03	283.6	0.489
9	A_EX03	443.9	0.498
10	A_EX04	354.7	0.492
11	A_EX04	398.9	0.539
12	A_EX05	234.7	0.506
13	A_EX07	309.9	0.485
14	A_EX08	329.1	0.480
15	A_EX11	159.2	0.546
16	A_EX13	173.2	0.560
17	A_EX14B	275.5	0.547
18	B-EX07	266.4	1.015
19	B-EX09	166.2	1.065
20	B-EX10	133.6	1.180
21	B-EX11	148.2	1.176
22	B-EX13	453.6	1.020
23	B-EX5	196.9	0.948
24	B_EX01	300.7	0.975
25	B_EX01	389.5	0.984
26	B_EX02	363.5	0.995
27	B_EX02	435.1	0.929
28	B_EX03	318.6	1.008
29	B_EX03	226.8	0.980
30	B_EX04	242.8	0.658
31	B_EX04	178.2	1.029
32	B_EX08	346.9	1.048
33	B_EX12	408.9	1.012
34	CP01	127.5	1.097
35	CP02	153.8	0.999
36	CP03	214.9	0.843
37	CP04	250.1	0.950
38	CP05	291.0	0.982
39	CP06	338.0	1.064
40	CP07	426.0	0.882
41	CP08	462.1	0.992
42	CP09	472.5	1.121

A: Comparison of peaks between standard and patient probes – red: mean of all samples tested, blue: patient. B: table showing relative signals for each probe in the MRC Holland GRIN2A/GRIN2B MLPA kit in the last column (patient sample compared to mean of all samples tested); normal values are defined between 0.7 and 1.3 and are shown in. white background, abnormal values are highlighted in grey. C: x axis: length in base-pairs for each probe, y axis: relative peak ratio for patient sample. Two horizontal grey lines demarcate the area for normal relative peak ratios (0.7 – 1.3). Normal values are depicted in green, abnormal values in red.

4.5 Clinical Features of GOSH *GRIN2A*-LKS Patients

The clinical features of all patients with genetic findings in the GOSH LKS cohort are summarized in the **Appendix**.

To investigate whether any clinical features are specifically associated with *GRIN2A* variants in LKS, we looked to see if there were any significant phenotypic differences between *GRIN2A*-positive and *GRIN2A*-negative individuals.

4.5.1 Speech Phenotype

Although this was not formally examined with standardized tests, a review of videos from speech and language assessments suggest that *GRIN2A*-positive LKS patients may have a distinct, recognizable speech phenotype. From observation, compared to *GRIN2A*-negative LKS patients, *GRIN2A*-positive patients tended to have milder difficulties with pitch and prosody. However, their speech is often hesitant with long pauses, and they often had significant difficulty with multiple repetitions and putting syllables in the correct order. These speech difficulties became more apparent with longer word length or phrases. These are all features consistent with oral dyspraxia. It is possible that *GRIN2A*-positive LKS patients may have more of a dyspraxic speech phenotype compared to *GRIN2A* negative LKS patients. A recent study comparing *GRIN2A* mutation-positive EASD individuals with normal control subjects reported similar findings (Turner et al., 2015).

4.5.2 Other clinical variables

Phenotypic comparison between *GRIN2A*-positive and *GRIN2A*-negative patients within the GOSH LKS cohort is summarized in **Table 4-5**. Statistical analysis suggests that *GRIN2A*-positive LKS patients were significantly more likely to have a prior history of speech and language delay (p=0.023) and significantly less likely to have clinical seizures (p= 0.013) (Fisher's exact test).

One of the study limitations is that within the GOSH LKS cohort there are much fewer *GRIN2A*-positive than *GRIN2A*-negative individuals (7 *GRIN2A* positive vs 38 *GRIN2A* negative). Therefore, to achieve better statistical comparison, we combined our data

with that of all other LKS patients with *GRIN2A* genotype and clinical features reported in literature to date. This brought the total number of *GRIN2A*-positive LKS patients to 22 and the total number of *GRIN2A* negative patients to 53 (**Table 4-6**).

The clinical details of patients extracted from literature are presented in **Table 4-7 and Table 4-8.** There were a few limitations to putting this data together accurately due to inevitable non-uniformity in the clinical features reported. Some papers reported only "age at seizure onset" or "age at onset" instead of "age at speech and language regression". For this analysis, it was assumed that these reported ages would be close to the age of regression for these individuals. Secondly, all papers reported language outcomes differently. All patients in the *GRIN2A* – positive group in the GOSH LKS cohort, recovered some level of language; none were left with no functional language. As such, for the category "language recovery", only individuals left with no functional speech were classified as having no recovery. Thirdly, not all studies reported on the same clinical features I collected for my study. As such, there were variables with missing data. Individuals with missing data for a variable were excluded from analysis for that variable, as indicated in **(Table 4-6).**

Analysis of the larger combined cohort now demonstrates that there is no significant difference in likelihood of clinical seizures between *GRIN2A*-positive and *GRIN2A*-negative individuals. However, the new dataset suggests that *GRIN2A*-positive LKS individuals are statistically more likely to have a positive family history (p= 0.021), and prior delay in speech development (p= 0.029). They are also less likely to have significant behavioural difficulties (p= 0.033). Even within the GOSH LKS cohort, the proportion of movement disorders in *GRIN2A*-positive LKS patients was more than twice that of *GRIN2A*-negative patients. In the larger combined dataset, this reaches statistical significance. Indeed, within the GOSH cohort, testing with the Movement Assessment Battery for Children (Brown and Lalor, 2009) has demonstrated that children with *GRIN2A* mutations may have motor dyspraxia.

The appearance of oromotor and motor dyspraxia in *GRIN2A*-positive individuals may reflect the fact that in addition to the cerebral cortex, *GRIN2A* is also relatively highly expressed (and may have key functional roles) in both the cerebellum and basal ganglia **(Figure 4-1)**.

Table 4-5: Phenotypic comparison between *GRIN2A*-positive (n=7) and *GRIN2A*-negative (n=38) individuals in the GOSH LKS cohort

Case	Variant	Inheritance	FHx	Speech delay before regression	Age at Regression	Seizures	Language Recovery	Below Ave non-verbal IQ	Behavioural difficulties	Motor Difficulties
79	c.1553G>A; p.Arg518His	De novo	No	No	5y6m	Yes	Yes	Yes	No	Yes
11	c.1552C>T; p.Arg518Cys	Inherited,	Yes	Yes	8y10m	Yes	Yes	Yes	Yes	Yes
9	c.2041C>T p.Arg681*	Unknown	No	No	5y0m	No	Yes	Yes	Yes	No
49	c.1289_1290delGT; p.Val430Glufs*18	Inherited,	Yes	Yes	6y10m	Yes	Yes	No	No	Yes
67	c.1776_1777dupAA p.Ala593Lysfs*62	De novo	Yes	Yes	6y10m	No	Yes	Yes	Yes	No
95	c.3212_3221del p.His1071Leufs*33	Inherited,	Yes	Yes	3y6m	No	Yes	No	No	No
68	arr 16p13.2	De novo	No	Yes	5y4m	No	Yes	No	Yes	Yes
	GRIN2A positive Mean (range) or %		57.1%	71.4%	6y0m ± 1y8m (3y6m to 8y10m)	42.9%	100%	57.1%	57.1%	57.1%
	GRIN2A negative Mean(range) or %		39.5%	23.7%	4y9m ± 2y0m (1y6m to 11y10m)	89.5%	84.2%	42.9%	84.2%	23.7%
	p- value (<0.05: significant)		0.433*	**0.023***	0.133†	**0.013***	0.569*	0.699*	0.131*	0.168*

Ave: Average; FHx: Family history; IQ: intelligence quotient; * Fisher's exact test; †Independent T-test

Table 4-6: Phenotypic comparison between *GRIN2A*-positive (n=22) and *GRIN2A*-negative individuals (n=53) – GOSH data combined with data from literature reports

Genotype	FHx	Speech delay before regression	Mean Age at SLR/ Onset	Seizures	Language recovery	Below Ave non-verbal IQ	Behavioural. Difficulties	Motor difficulties
GRIN2A mutation positive N= 22	66.7% $N^{‡}$= 21	56.3% $N^{‡}$= 16	4y7m ± 1y9m (1y0m to 8y10m)	72.7%	94.1% $N^{‡}$=17	73.7% $N^{‡}$=19	56.3% $N^{‡}$= 16	61.5% $N^{‡}$= 13
GRIN2A mutation negative N= 53	35.8% N=53	23.7% $N^{‡}$= 38	4y10m ± 1y11m (1y6m to 11y10m)	88.7%	78.8% $N^{‡}$=52	45.3% N=53	85.4% $N^{‡}$= 41	23.7% $N^{‡}$= 38
p- value (<0.05: significant)	**0.021***	**0.029***	0.659†	0.163*	0.269*	0.059*	**0.033***	**0.019***

FHx: Family history; *Fisher's exact test; †Independent T-test; IQ: intelligence quotient; SLR: speech and language regression; N: number of patients; $N^{‡}$: where data is missing, this indicates the number of patients with data available for analysis

Table 4-7: Clinical features for *GRIN2A* positive LKS individuals reported in literature

Case Identifier	Variant	FHx	Speech delay before SLR	Age at SLR/onset	Seizures	Language Recovery	Below Ave non-verbal IQ	Behavioural difficulties	Motor Difficulties
EA85[1]	arr16p13.2; 16: 10,246,239-10,354,862	Yes	Yes	5y	Yes	No	No	Yes	NR
Family 3 301[2]	arr16p13.2; 16:9,915,756-9,915,815	Yes	No	5y*	Yes	Yes	NR	No	NR
Case 1 DZ98[2]	c.2797G>A; p.Asp933Asn	No	No	1y*	Yes	Yes	No	No	NR
Case 3-81[2]	c.2081T>C; p.Ile694Thr	No	No	2y6m*	Yes	Yes	Yes	Yes	NR
Case 5 DY29[2]	c.1642G>A; p.Ala548Thr	No	Yes	6y*	Yes	Yes	Yes	Yes	NR
Family 1 DZ29[2]	c.1123-2A>G	Yes	Yes	4y*	Yes	Yes	Yes	No	NR
BIII2[3]	c.2T>C; p.Met1Thr	Yes	No	3y6m*	Yes	NR	Yes	NR	NR
BrnIndex-2[4]	c.2041C>T; p.Arg681*	Yes	NR	3y6m	No	NR	Yes	NR	No
BrnIndex-3[4]	c.1007+1G>A	Yes	NR	4y†	Yes	NR	Yes	NR	No
Pt74-5[4]	c.692G>A; p.Cys231Tyr	Yes	NR	3y†	Yes	NR	Yes	NR	Yes
Patient 7[5]	c.1553G>A; p.Arg518His	No	NR	6y	No	Yes	Yes	Yes	NR
Patient[6]	c.2146G>A; p.Ala716Thr	Yes	No	3y	Yes	Yes	NR	No	Yes
Family 1[7]	c.2191G>A; p.D731N	Yes	NR	5y†	Yes	NR	NR	NR	NR
Patient 4[8]	c.2407G>T; p.Glu803*	NR	Yes	2y6m	Yes	Yes	Yes	NR	Yes
Patient 2[9]	c.2041C>T; p.Arg681*	Yes	NR	5y	Yes	Yes	Yes	Yes	Yes

Ave: average; FHx: family history; SLR: speech and language regression; IQ: intelligence quotient; m: months; NR: not reported; y: years. *Only "age at seizure onset" reported, assumed to be close to age at SLR; †only age at "onset" reported, assumed to be at/close to age at SLR. References: [1](Lesca et al., 2012); [2](Lesca et al., 2013); [3](Carvill et al., 2013b); [4](Lemke et al., 2013); [5](Conroy et al., 2014); [6](Fainberg et al., 2016); [7](Dyment et al., 2015); [8](von Stulpnagel et al., 2017); [9](Hausman-Kedem et al., 2020)

Table 4-8 Clinical features for *GRIN2A* – negative LKS individuals reported in the literature

Case Identifier	FHx	Speech delay before SLR	Age at SLR/onset	Seizures	Language Recovery	Below Ave non-verbal IQ	Behavioural difficulties	Motor Difficulties
1-a[1]	No	NR	3y*	Yes	No	No	NR	NR
2-a[1]	No	NR	5y*	Yes	Yes	Yes	NR	NR
3[1]	No	NR	5y6m*	Yes	No	Yes	NR	NR
4[1]	No	NR	3y*	Yes	No	Yes	NR	NR
5[1]	No	NR	4y*	Yes	Yes	No	NR	NR
6[1]	Yes	NR	4y6m*	Yes	Yes	Yes	NR	NR
8[1]	Yes	NR	6y6m*	Yes	Yes	No	NR	NR
9[1]	No	NR	2y6m*	Yes	No	No	NR	NR
10[1]	No	NR	4y*	Yes	Yes	No	NR	NR
11[1]	No	NR	6y6m*	Yes	Yes	No	NR	NR
12[1]	Yes	NR	4y*	Yes	Yes	No	NR	NR
13[1]	No	NR	5y*	No	Yes	No	NR	NR
19[2]	No	NR	5y4m	Yes	NR	Yes	Yes	NR
20[2]	No	NR	8y	No	Yes	No	Yes	NR
21[2]	Yes	NR	7y9m	Yes	No	Yes	Yes	NR

Ave: average; FHx: family history; SLR: speech and language regression; IQ: intelligence quotient; m: months; NR: not reported; y: years. *only age at "onset" reported, assumed to be close to age at SLR. References: [1](Conroy et al., 2014); [2](Pavlidis et al., 2019)

4.6 Functional investigation of *GRIN2A* variants

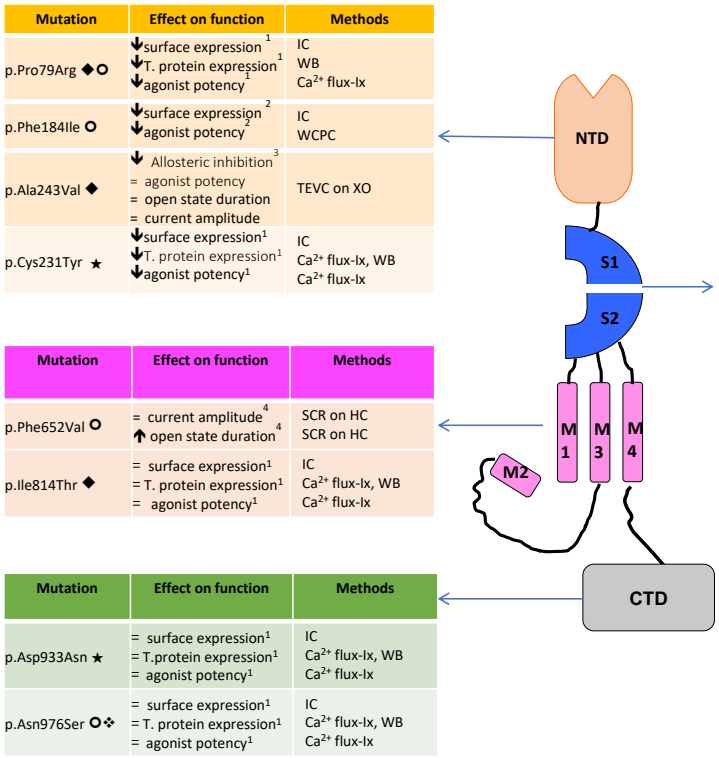
When a genetic aetiology is identified for a given disorder, deciphering the effects of causative genetic variants on protein function offers precious insight to mechanisms that give rise to disease. This, in turn, affords the opportunity for the design of more efficacious, targeted treatment options.

As mentioned briefly in **Section 4.3.2,** functional investigations have been undertaken on a wide range of reported *GRIN2A* missense mutations involving different protein domains. In addition to EASD, these include investigations that encompass a wide range of the different phenotypes associated with *GRIN2A* including other epileptic encephalopathies, schizophrenia and movement disorders.

Mutant protein has been shown to affect normal NMDA receptor function through alterations in total protein levels, protein localization (surface expression), allosteric modulation, sensitivity to voltage dependent Mg^{2+} block, agonist potency, duration of channel opening/closing, current amplitudes, and deactivation rates **(Figure 4-8).**

Five of the seven *GRIN2A* mutations identified in our GOSH LKS cohort (1 whole gene deletion, 3 intragenic frameshift deletions or insertions and 1 nonsense mutation) would be expected to have deleterious effects on protein synthesis, leading to either a truncated protein product or complete absence of the protein. To elucidate the mechanisms through which the 2 remaining rare missense variants, p.Arg518His and p.Arg518Cys lead to LKS, we investigated their effect on gene expression, total protein levels, protein localization, NMDA receptor structure and NMDA receptor function.

Figure 4-8 Results of functional investigations of previously reported *GRIN2A* mutations in EASD



Mutation	Effect on function	Methods
p.Pro79Arg ◆O	↓surface expression[1] ↓T. protein expression[1] ↓agonist potency[1]	IC WB Ca^{2+} flux-Ix
p.Phe184Ile O	↓surface expression[2] ↓agonist potency[2]	IC WCPC
p.Ala243Val ◆	↓ Allosteric inhibition[3] = agonist potency = open state duration = current amplitude	TEVC on XO
p.Cys231Tyr ★	↓surface expression[1] ↓T. protein expression[1] ↓agonist potency[1]	IC Ca^{2+} flux-Ix, WB Ca^{2+} flux-Ix

Mutation	Effect on function	Methods
p.Phe652Val O	= current amplitude[4] ↑ open state duration[4]	SCR on HC SCR on HC
p.Ile814Thr ◆	= surface expression[1] = T. protein expression[1] = agonist potency[1]	IC Ca^{2+} flux-Ix, WB Ca^{2+} flux-Ix

Mutation	Effect on function	Methods
p.Asp933Asn ★	= surface expression[1] = T.protein expression[1] = agonist potency[1]	IC Ca^{2+} flux-Ix, WB Ca^{2+} flux-Ix
p.Asn976Ser O❖	= surface expression[1] = T. protein expression[1] = agonist potency[1]	IC Ca^{2+} flux-Ix, WB Ca^{2+} flux-Ix

Mutation	Effect on function	Methods
p.Cys436Arg ❖	↓ agonist potency[1,5] ↑ agonist potency[5] ↓ surface expression[1]	Ca^{2+} flux-Ix TEVC on XO[5] IC
p.Gly483Arg O	↓surface expression[1,5] ↓agonist potency[1,5]	Ca^{2+} flux-Ix, BL[5] Ca^{2+} flux-Ix, TEVC on XO[5]
p.Arg504Trp O	↑ agonist potency[5]	TEVC on XO[5]
p.Arg518His ★	= current amplitude[4] ↑ open state duration[4] ↓ surface expression[2,5] ↓ agonist potency[2,5]	SCR on HC[4] SCR on HC[4] IC[2], BL[5] WCPC[2], TEVC on XO[5]
pThr531Met O	↑ open state duration[6] ↓agonist potency[5]	SCR on COS-7 TEVC on XO[5]
p.Lys669Asn O	↑ agonist potency[5]	TEVC on XO[5]
p.Ile694Thr ★	↓ agonist potency[5]	TEVC on XO[5]
p.Pro699Ser ◆	↑ agonist potency[5]	TEVC on XO[5]
p.Met705Val ◆	↓surface expression[1] ↓T. protein expression[1] ↓agonist potency[1,5]	IC[1] Ca^{2+} flux-Ix[1], WB[1] Ca^{2+} flux-Ix[1], TEVC on XO[5]
p.Glu714Lys O	↓surface expression[1] ↓T. protein expression[1] = agonist potency	IC Ca^{2+} flux-Ix, WB TEVC on XO[5]
p.Ala716Thr ★❖	↓ agonist potency[5] = agonist potency	TEVC on XO[5] WCPC[2]
p.Ala727Thr ◆	↓ agonist potency[5]	TEVC on XO[5]
p.Asp731Asn ★❖	↓ current amplitude[6] ↓ open state duration[6] ↓ surface expression[1,5] ↓ agonist potency[1,5,6] ↑ allosteric inhibition[6]	WCPC[6] TEVC on XO[6] IC[1], BL[5], Ca^{2+} flux-Ix[1],TEVC on XO[5,6] TEVC on XO[6]
p.Val734Leu ◆	↓ agonist potency[5]	TEVC on XO[5]
p.Lys772Glu ❖	↓ agonist potency[5]	TEVC on XO[5]

Legend
◆Childhood epilepsy with centrotemporal spikes)
❖Intermediate epilepsy aphasia disorder
OEpilepsy with continuous spike-waves in slow wave sleep
★Landau Kleffner Syndrome
↑ increased
↓decreased
=: equivalent/no effect on protein

BL: Beta-lactamase assay; Ca^{2+} influx Ix: Calcium influx imaging; CTD: carboxyl-terminal domain; HC: HEK-293 cells; IC: immunocytochemistry; M1-M4: transmembrane domains; NTD: N-terminal domain; S1 and S2: subunits of Ligand binding domain; SCR: single channel recording; TEVC: two electrode voltage clamp; WB: Western blot; WCPC: whole cell patch clamping, XO: *Xenopus Laevis* oocytes. 1:(Addis et al., 2017); 2: (Sibarov et al., 2017); 3: (Lemke et al., 2013); 4: (Lesca et al., 2013); 5: (Swanger et al., 2016); 6: (Gao et al., 2017)

4.7 *In vitro* over-expression cell model

I utilized an over-expression Human Embryonic Kidney (HEK-293) cellular model to study the effect of the two *GRIN2A* missense variants, identified in the GOSH LKS cohort, on gene expression, protein expression and protein localization.

I undertook site-directed mutagenesis to generate plasmids harbouring *GRIN2A* variants c.1552C>T and c.1553G>A, using methods described in **Chapter 2, Section 2.3.6.**

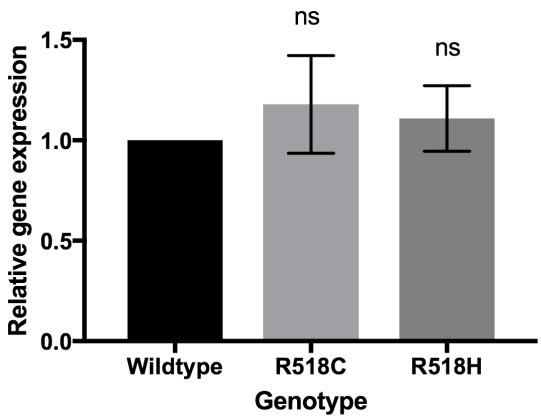
After site-directed mutagenesis, mutant plasmids were validated by Sanger sequencing. Lipofectamine-2000 (Thermo-Fisher) was then used to co-transfect HEK-293 cells with the following combination of constructs: (i) N1-wild-type and N2A-wild-type; (ii) N1-wild-type and N2A- Arg518Cys; and (iii) N1-wild-type and N2A-Arg518His **(Chapter 2, Section 2.3.7).**

4.8 Effect on gene and protein expression

24 hours after transfection, reverse-transcription polymerase chain reaction (RT-PCR) and immunoblotting were performed on whole- cell lysates extracted from transfected HEK-293 cells to compare wild-type (WT) and mutant N2A gene and protein expression **(Chapter 2, Sections 2.3.8 and 2.3.9).**

RT-PCR performed in triplicate revealed no significant difference in *GRIN2A* gene expression when comparing N2A-WT with N2A-Arg518Cys, and N2A-Arg518His transfected cells **(Figure 4-9).**

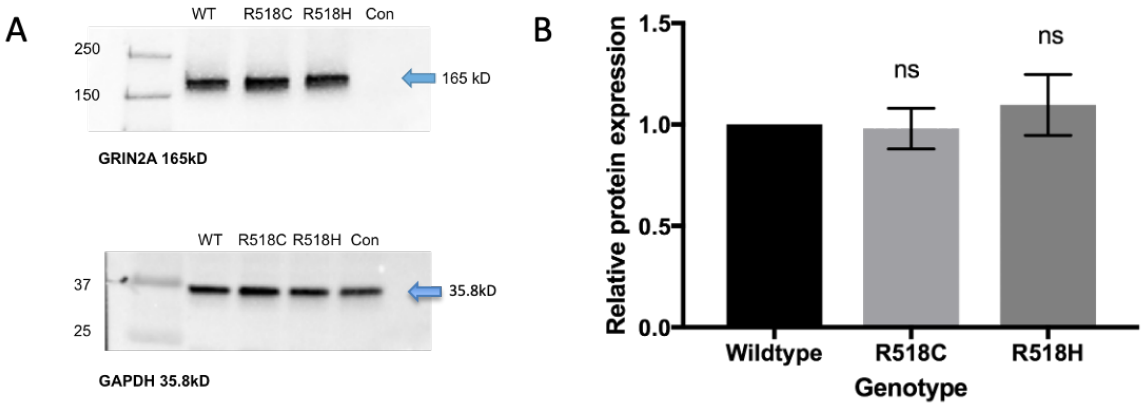
Figure 4-9: RT-PCR Results



Relative gene expression (fold-change) for *GRIN2A* variants R518C and R518H normalized to wild-type (data derived from triplicate measurements). ns= not significant. Statistics undertaken using one-way ANOVA in Prism 7.0 (GraphPad).

Results of 3 different immunoblotting experiments from 3 independent transfections indicated that there was no significant difference in N2A protein expression between N2A- WT, N2A-Arg518Cys and N2A-Arg518His transfected cells **(Figure 4-10).**

Figure 4-10: Immunoblotting Results



(A) Representative Western blot of HEK-293 whole cell lysates probed with anti-N2A antibody (top) and anti-GAPDH antibody as a loading control (bottom). Con = untransfected control HEK-293 cells. (B) Plot of N2A protein expression for N2A-Arg518Cys and N2A-Arg518His relative to wild-type- normalized to GAPDH (average results from 3 independent transfections). ns= not significant. Statistics undertaken using one-way ANOVA in Prism 7.0 (GraphPad).

4.9 Effect on protein localization

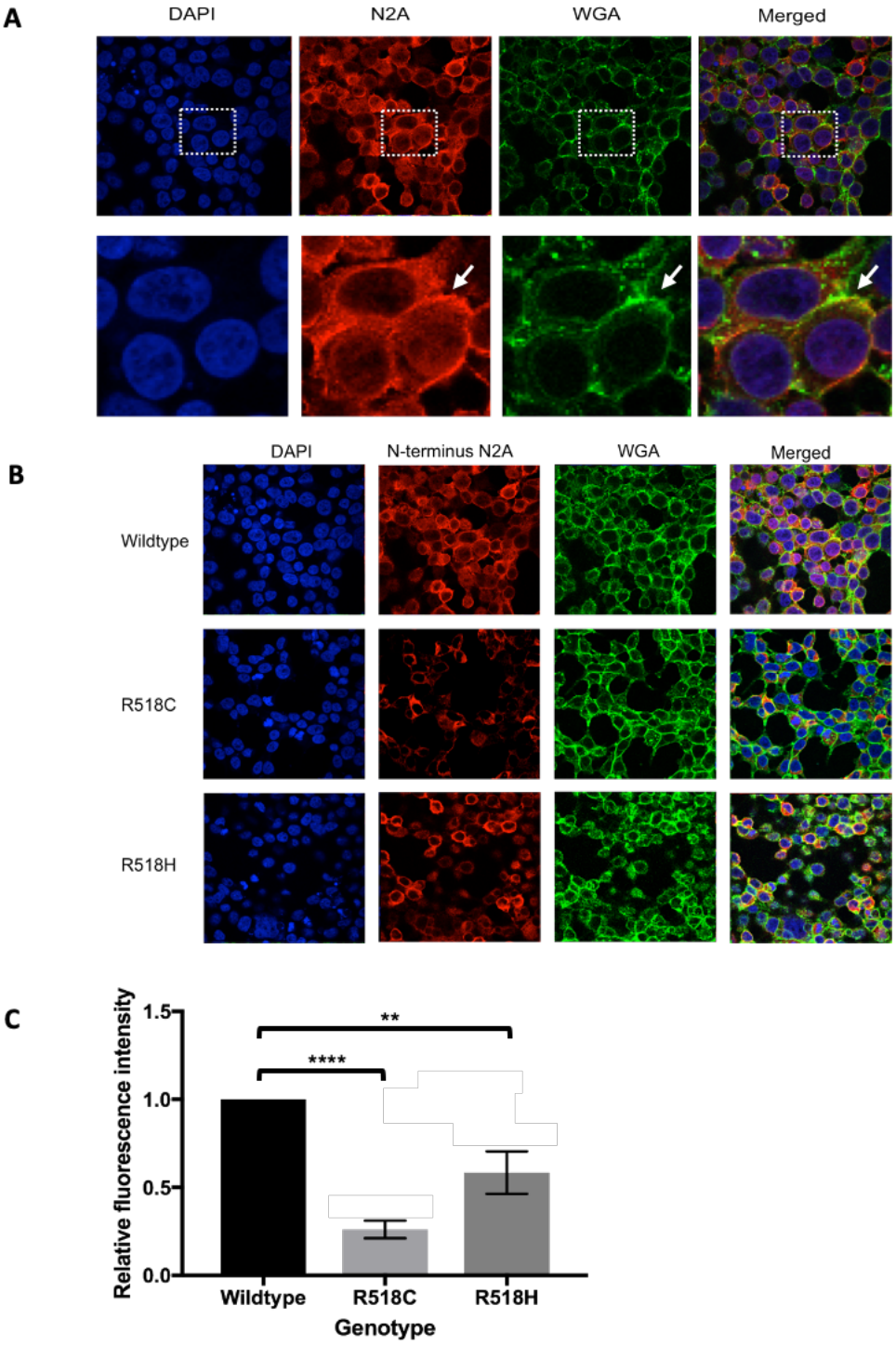
My experiments showed that p.Arg518Cys and p.Arg518His have no significant effect on both gene and total protein expression, I therefore performed further investigations to see if these mutations would affect protein localisation.

HEK-293 cells were fixed 24 hours after transfection and immunostained under non-permeabilised conditions **(Chapter 2, Section 2.3.10).** This maintains the integrity of the cell membrane, restricting antibody access to intra-cellular antigens. The resulting slides were viewed under the Zeiss LSM 710 inverted confocal microscope and five Z-stack images were taken for each genotype at x63 magnification.

Images were analysed using Fiji software and a macro written by Dr Dale Moulding, UCL, Great Ormond Street Institute of Child Health. To look at cell- surface expression of N2A for each genotype, the intensity of N2A immunofluorescence co-localizing with the membrane surface marker, wheat-germ agglutinin (WGA), was averaged to cell count, for N2A-WT, N2A-Arg518Cys and N2A-Arg518His transfected cells **(Chapter 2.3.10).**

The steps above were repeated for three independent transfections.

Figure 4-11: Protein Localization results

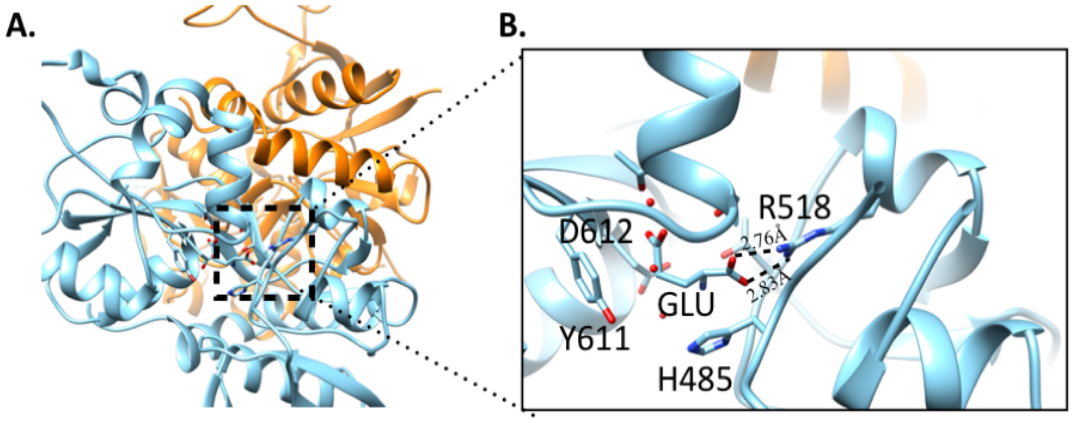


A: Representative confocal microscopy image of HEK-293 cells co-transfected with wildtype N1 and wildtype N2A (x63) enlarged to show co-localization of surface N2A with membrane marker wheat germ agglutinin (WGA) (arrow). B: Comparison of confocal microscopy images from HEK-293 cells transfected with wildtype N1 and either N2A-wildtype, N2A- Arg518Cys, or N2A- Arg518His (x63). C: Quantification of relative fluorescence intensity of surface N2A-Arg518Cys and N2A-Arg518His compared to N2A-wildtype (average measurements from 3 independent transfections). ***p*= <0.005, *****p*= <0.0001. Statistics undertaken using one-way ANOVA in Prism 7.0 (GraphPad).

4.10 Effect on NMDA receptor structure

Arg518 is known to be a highly conserved residue across all NMDA receptor subunits and is involved in agonist binding (Traynelis et al., 2010). Homology modelling to determine the structure-function properties of *GRIN2A* missense variants was performed by our collaborators, Dr Maya Topf at the Institute of Structural and Molecular Biology, UCL Birkbeck College, and Dr Sony Malhotra at the Department of Biochemistry, University of Cambridge **(Chapter 2, Section 2.3.11).** They concluded that the replacement of the highly conserved arginine, which interacts with the acidic group of glutamate in the ligand binding site, by either cysteine or histidine, is likely to affect the agonist binding kinetics of N2A **(Figure 4-12).**

Figure 4-12: Homology modelling



Structure model of the NMDA receptor protein (N1 in orange and N2A in blue). Enlarged view shows R518 within the agonist binding domain and its' interaction with glutamate.

4.11 Electrophysiology experiments

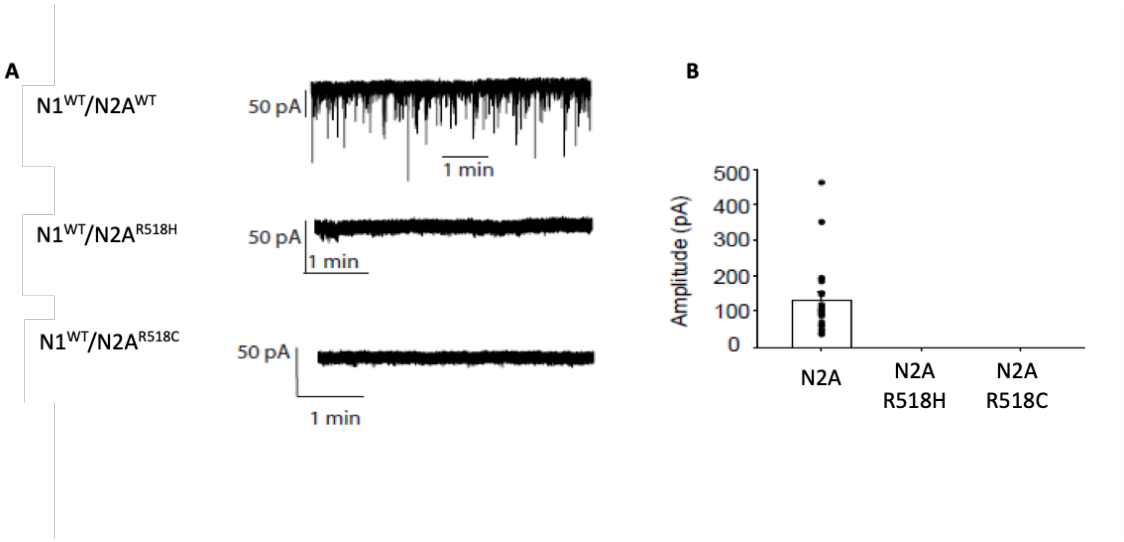
We sought to determine whether p.Arg518His and p.Arg518Cys affected the properties of excitatory post- synaptic currents (EPSCs) mediated by N1/N2A receptors in artificial synapses. Electrophysiology experiments were performed by Dr Joe Lynch and Professor Robert Harvey, at the University of the Sunshine Coast, Australia.

Rat cortical neurons and HEK-293 cells transfected with either wild-type or mutant *GRIN2A* were co-cultured to create 'artificial' synapse preparations. Both Arg518Cys

and Arg518His were tested and compared to wild-type in standard dose-response experiments.

In control experiments, EPSCs mediated by cells transfected with wild-type N1/N2A receptors, were recorded in 40/50 cells over a total of 4 transfections (**Figure 4-13**). By contrast, there was no evidence for EPSCs mediated by either N1/N2A-Arg518Cys or N1/N2A-Arg518His transfected cells in a total of 25 cells each spread out over 4 transfections (i.e. EPSCs recorded in 0/25 cells for each mutant receptor). Similarly, there was no evidence for whole-cell glutamate activated currents in HEK293 cells co-transfected with N1/N2A-Arg518Cys or N1/N2A-Arg518His receptors, indicating loss of agonist potency.

Figure 4-13: Electrophysiology results



A: Properties of EPSCs mediated by di-heteromeric N1/N2A- WT, N1/N2A- Arg518His and N1/GluN2A-Arg518Cys NMDARs in artificial synapses. **B:** Mean amplitude of EPSCs from cells expressing the indicated subunit combinations. n values for each observation were N1/N2A-WT (n=22), N1/N2A -Arg518His (n=12) and N1/N2A- Arg518Cys (n=18).

4.12 Summary

In this chapter, I have described the spectrum of *GRIN2A* mutations reported in individuals with LKS and related EASD. To date 51 different mutations have been reported in association with EASD, accounting for 8-20% of patients. The frequency of *GRIN2A* mutations within the GOSH LKS cohort (15.5%) is consistent with previous literature reports, confirming that LKS is likely to be genetically heterogeneous. Like

many epilepsy genes, *GRIN2A* mutations are associated with incomplete penetrance, variable expressivity and phenotypic pleiotropy.

My study has highlighted that *GRIN2A*-positive LKS patients may have a recognizable dyspraxic speech pattern. Furthermore, in comparison to *GRIN2A* negative LKS patients, *GRIN2A* positive LKS patients are more likely to have a positive family history, a prior history of speech and language delay, and movement difficulties. They are less likely to have significant behavioural difficulties.

When *GRIN2A* mutations were first described in EASD and other developmental epileptic encephalopathies in 2013, early functional investigations performed on 5 mutations (p.A243V, p.R518H, p.N615K, p.F652V and p.L812M), suggested that *GRIN2A* mutations had an overall gain of function effect *in vitro* (Strehlow, 2015a). However, subsequent investigations have since revealed that the majority of *GRIN2A* mutations associated with EASD have an overall *loss-of-function* effect (**Figure 4-8**).

One of the missense mutations discovered in our cohort, p.Arg518His, was previously reported in 2 patients with LKS (Lesca et al., 2013, Conroy et al., 2014). Functional investigations undertaken by Lesca et al in 2013 using single channel recording on transfected HEK-293 cells concluded that this mutation resulted in increased channel open duration, implying a gain of function effect (Lesca et al., 2013). Contrary to this, subsequent work has demonstrated reduced surface expression and agonist potency, suggesting a likely overall loss of function effect. In 2016, with beta-lactamase assays on HEK-293 cells and two-electrode voltage clamp experiments on *Xenopus Laevis* oocytes, Swanger et al reported that this variant resulted in a 50% reduction in N2A membrane expression and virtually no detectable current response (Swanger et al., 2016). In 2017, using immunocytochemistry investigations and whole-cell patch clamping on transfected HEK-293 cells, Sibarov et al similarly reported a 33% reduction in mutant N2A surface expression and drastically reduced current response (Sibarov et al., 2017).

The results of our investigations on p.Arg518His are consistent with Swanger et al's and Sibarov et al's results, reliably confirming that this variant results in reduced NMDA receptor surface expression (57% of normal) and agonist potency, despite no significant change in gene or total protein expression.

The other missense variant discovered in our cohort, p.Arg518Cys, affects the same amino acid and has not been previously reported in literature. Our investigations reveal that this variant, inherited from an unaffected father, has similar functional consequences to those observed for p.Arg518His, with even lower membrane surface expression (23% of normal) and no detectable current response.

The results of *GRIN2A*-related functional work have enabled us to draw two important conclusions. Firstly, *GRIN2A* mutations dysregulate NMDA receptor function via several different mechanisms including effects on allosteric inhibition, channel opening duration, agonist binding and deactivation times. It is plausible that a given mutation may induce a gain of function effect via one mechanism, yet bring about opposing loss of function effects via other mechanisms (Swanger et al., 2016). As such, when evaluating the net effects of a particular mutation, it is important to comprehensively analyse several facets of NMDA receptor function to better understand its overall impact. Secondly, it is possible that both enhanced and impaired N2A function may bring about relatively similar phenotypes. Consequently, we cannot deduce underlying mechanisms from phenotype alone, and therapeutic strategies will need to be tailored to specific mutations, after careful functional evaluation.

5 Chapter 5: Molecular Genetic Investigation of *GRIN2A*-negative LKS Part 1 – Panel Analyses

5.1 GOSH LKS Cohort

As *GRIN2A* only accounts for 8-20% of LKS/related EASD, it is likely that other genes also cause these phenotypes. In order to identify candidate genes, we performed familial triome whole exome sequencing (WES)/whole genome sequencing (WGS) for all the families in the GOSH LKS cohort who screened negative for *GRIN2A* mutations.

Detailed methods for WES/WGS are described in **Chapter 2, 2.3.4**.

In total, of the 92 patients on the GOSH LKS database, we received DNA for 62 families. Seven of these tested positive for *GRIN2A* mutations. 1 patient was discovered to have NMDA receptor antibodies, and was excluded from further genetic analysis. As such, DNA from a total of 54 families were sent for WES/WGS investigations. When this project first started in 2015, the first batch of *GRIN2A* negative patients were sent for familial WES; (11 triomes, and 2 families for whom only maternal and proband DNA samples were sent, as paternal DNA was unavailable). As WGS became increasingly accessible, subsequent batches of *GRIN2A*-negative families were sent for familial WGS (27 triomes, 3 families for whom only maternal and proband DNA samples were sent, as paternal DNA was unavailable, and 1 single proband as parental DNA samples were unavailable). DNA from 10 families was acquired towards the end of the project. Due to budget constraints, DNA from these families was sent only for proband whole exome sequencing **(Figure 5-1).**

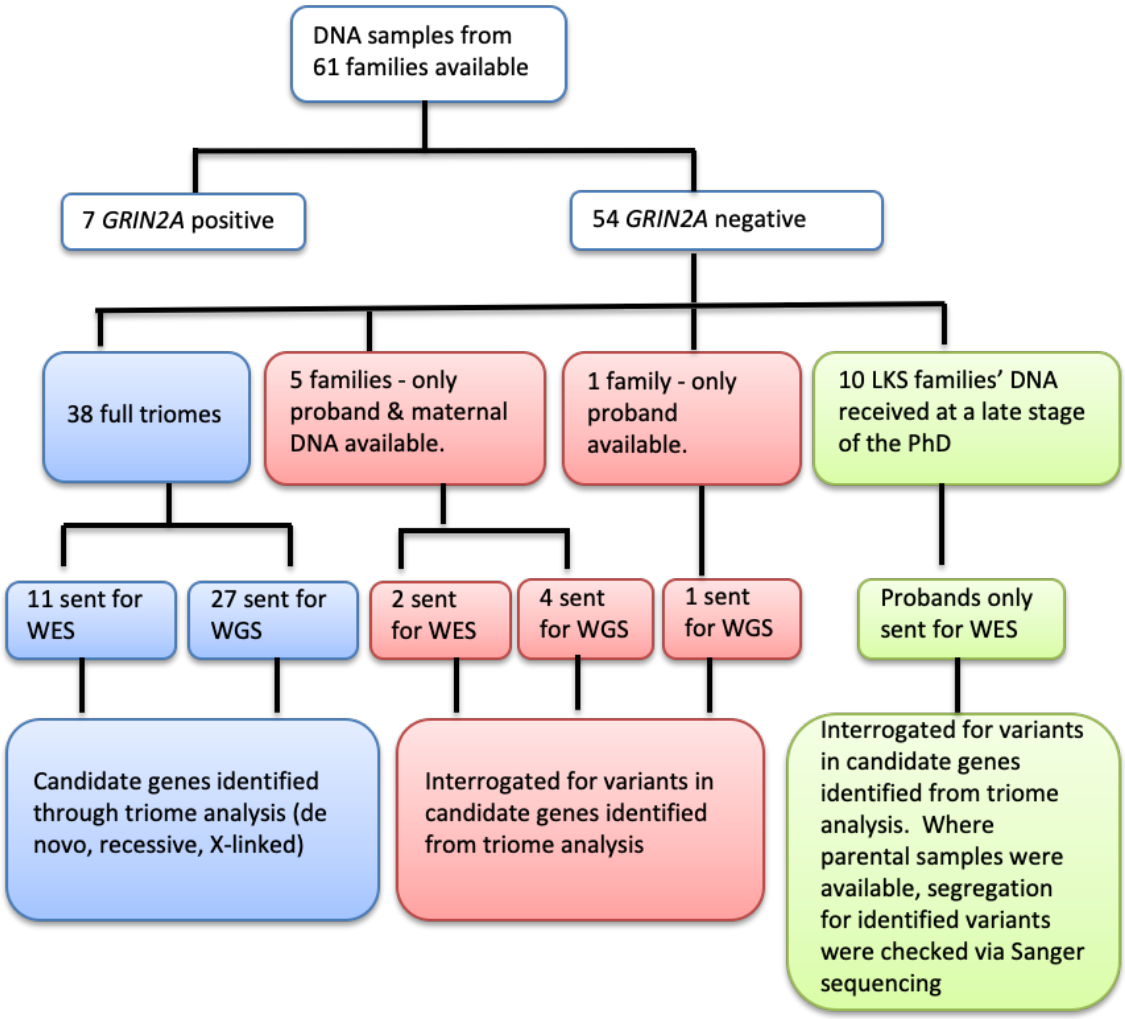
One of the triomes included an affected father and son (Family 3) and 1 family included 2 affected siblings (Family 45). Therefore, in total, I had 56 patient (case) samples and 80 parental (control) samples for analysis.

WES/WGS data was systematically analysed (**Chapter 5.3**). Most significant variants in candidate genes were verified with Sanger sequencing. Some variants, identified towards the end of this study could not be verified by Sanger sequencing in time for the writing of this thesis due to laboratory restrictions brought about by the COVID-19 pandemic. For these variants, raw sequence .bam files and Integrated Genomics Viewer (IGV) software (http://software.broadinstitute.org/) were used to check coverage and

read depth to verify the reliability of the variant call. Variants within poorly sequenced regions were disregarded.

All *GRIN2A* negative individuals who underwent whole genome sequencing were screened for genome wide copy number variants (n=32) via LUMPY performed by bioinformaticians at GOSgene as described in **Chapter 2, Section 2.3.5** (Layer et al., 2014). 5 of these 32 individuals also underwent single nucleotide polymorphism microarray testing at Great Ormond Street hospital diagnostic laboratory as part of their clinical work-up. For the 22 probands who underwent whole exome sequencing, copy number variant (CNV) screening via LUMPY was not possible. 1 of these cases (case 43) had CNV testing via an external diagnostic laboratory. Due to budget constraints, the remaining 21 probands have yet to have CNV screening.

Figure 5-1 Summary of genetic investigations for GOSH LKS Cohort



5.2 Results: Copy Number Variant Screening

Microarray testing for Case 43 uncovered a 3.9Mb duplication at the distal tip of the short arm of chromosome 1: 1p36.33p.36.32(751796-4678966) x 3 ish der(14)t(1;14)(p.36.32;q32.33). Apart from the duplication, no other significant imbalances were detected. This information for Case 43 only became available after we sent this proband's DNA for WES. Very few reports of similar duplications are present in literature (Marquet et al., 2017). As it was uncertain if this patient's phenotype (summarised in the **Appendix**) could be entirely explained by this duplication, I still proceeded with WES analysis for this family. No significant copy number variants were identified in the rest of the GOSH *GRIN2A*- negative LKS cohort.

5.3 WES/WGS Search Strategy

A systematic, step-wise search strategy was employed to identify candidate genes in the GOSH LKS cohort. Using Qiagen's Ingenuity variant analysis platform, a number of different analyses were performed as follows:-

(1) Panel analysis to identify variants in known epilepsy genes, i.e. genes that have previously been established to cause epilepsy syndromes **(Chapter 5.4).**

(2) Panel analysis to identify variants in genes that have previously been proposed to have an association with LKS/EASD, i.e. genes previously implicated in these phenotypes but where there is yet insufficient evidence to determine causality **(Chapter 5.5**).

(3) Individual familial triome analysis, in order to identify *de novo* dominant, autosomal recessive (homozygous or compound heterozygous), and X-linked (in male probands) variants for each family **(Chapter 6.1)**.

(4)Given that the majority of reported *GRIN2A* mutations within LKS/EASD were inherited from unaffected parents, to search for candidate genes with incomplete penetrance, I performed an analysis to identify genes in which autosomal dominant-inherited variants occurred frequently (3 or more families) within the LKS cohort **(Chapter 6.4).**

5.4 Panel Analysis for Reported Epilepsy Genes

5.4.1 Methods

A panel of previously reported epilepsy genes was assembled using both the Online Mendelian Inheritance in Man (OMIM) database and through literature review **(Table 5-1)**. Genes associated with disorders with phenotypic overlap with LKS were selected. Genes associated predominantly with metabolic disorders or structural malformations were excluded as these do not match the classical phenotype for LKS.

All WES/WGS data was uploaded in the form of variant call files to Qiagen's Ingenuity Variant Analysis (QIVA) platform. A set of pre-determined filters was used to remove low confidence variants, common variants, and likely benign variants **(Table 5-2)**. The desired gene panel was then uploaded onto the QIVA platform and the program was asked to filter for variants within the genes of interest. Following this, the resultant variant list was exported into a Microsoft Excel spreadsheet for further manual analysis.

During manual analysis, variants that were not detected in any probands, and only found in unaffected parents were discarded. Single allelic variants in genes reported to cause autosomal recessive epilepsy were also disregarded.

The frequency of each variant in control population databases including gnoMAD, ExAC, 1000 genomes project, ESP, and dbSNP was checked (introduced in **Table 5-2**). This was facilitated by the use of Alamut Visual software (https://www.interactive-biosoftware.com). Frequency thresholds were set according to known inheritance patterns for each gene (higher frequency thresholds of up to 0.1% were allowed for genes with autosomal recessive inheritance, and lower thresholds of 0.05% were set for genes with autosomal dominant inheritance). Identified heterozygous variants with homozygous occurrence in any control population database were excluded.

From Alamut Visual software, it was noted if a given variant had previously been submitted to the ClinVar database, a freely accessible archive of variants previously identified in patient samples, submitted by investigators, along with their clinical significance (https://www.ncbi.nlm.nih.gov/clinvar).

Each variant's Combined Annotation Dependent Depletion (CADD) score was determined (Kircher et al., 2014). Variants with a CADD score of less than 20 were discarded, unless they were frame-shift or splice-site variants **(Table 5-3).**

Additionally, each missense variant was put through five other in-silico prediction algorithms to determine the likelihood of pathogenicity. This was also partly facilitated by the use of Alamut Visual software (https://www.interactive-biosoftware.com). The in-silico prediction algorithms used are described in **Table 5-3**. Only variants that were predicted to be pathogenic by 3 or more different in-silico prediction algorithms were kept.

For variants that may be located within splice sites, splicing defect predictions from the splice site algorithms, Neural Network Splice (NNSplice), Splice Site Finder- like (SSF), and MaxEntScan (introduced in **Table 5-3**) were derived using Alamut Visual software (https://www.interactive-biosoftware.com). For each algorithm, Alamut Visual provides a calculation of percentage difference from the wild type sequence score to the variant sequence score. As recommended by previous literature, a combination of a percentage difference of 15% or more for MaxEntScan **and** 5% or more for NNSplice and SSF was taken to imply a likely splice site disruption (Houdayer et al., 2012, Tang et al., 2016).

Finally, the American College of Medical Genetics and Genomics guidelines were used to help interpret sequence variants (Richards et al., 2015). The criteria stipulated by these guidelines are presented in **Figure 5-2** and **Table 5-4**.

Table 5-1 Panel of previously reported epilepsy genes

Gene (MIM number)	Phenotype (MIM number)	Mode of inheritance	References
ARX (300382)	EIEE 1 (308350)	XLR	(Mirzaa et al., 2013, Kato et al., 2004, Tapie et al., 2017)
CDKL5 (300203)	EIEE 2 (300672)	XLD	(Kalscheuer et al., 2003, Weaving et al., 2004, Tao et al., 2004, Bahi-Buisson et al., 2008, Fehr et al., 2013, Kilstrup-Nielsen et al., 2012)
SLC25A22 (609302)	EIEE 3 (609304)	AR	(Molinari et al., 2005, Molinari et al., 2009, Poduri et al., 2013)
STXBP1 (602926)	EIEE 4 (612164)	AD	(Saitsu et al., 2008, Carvill et al., 2014)
SPTAN1 (182810)	EIEE 5 (613477)	AD	(Tohyama et al., 2008, Nonoda et al., 2013, Syrbe et al., 2017)
SCN1A (182389)	EIEE 6 (607208)	AD	(Fujiwara et al., 2003, Harkin et al., 2007, Carranza Rojo et al., 2011, Brunklaus et al., 2014)
KCNQ2 (602235)	EIEE 7 (613720)	AD	(Dedek et al., 2003, Borgatti et al., 2004, Weckhuysen et al., 2012, Saitsu et al., 2012, Bassi et al., 2005, Kato et al., 2013)
ARHGEF9 (300607)	EIEE 8 (300607)	XLR	(Harvey et al., 2004, Shimojima et al., 2011)
PCDH19 (300460)	EIEE 9 (300088)	X-linked	(Smith et al., 2018, Ryan et al., 1997, Scheffer et al., 2008, Depienne et al., 2009, Jamal et al., 2010, Pederick et al., 2018)
PNKP (605610)	EIEE 10 (613402)	AR	(Shen et al., 2010, Poulton et al., 2013)
SCN2A (182390)	EIEE 11 (613721)	AD	(Kamiya et al., 2004, Ogiwara et al., 2009, Howell et al., 2015, Sanders et al., 2018)
PLCB1 (607120)	EIEE 12 (613722)	AR	(Kurian et al., 2010, Poduri et al., 2012, Ngoh et al., 2014, Schoonjans et al., 2016)
SCN8A(600702)	EIEE 13 (614558)	AD	(Veeramah et al., 2012, Carvill et al., 2013a, Ohba et al., 2014, Butler et al., 2017)
KCNT1(608167)	EIEE 14 (614959) NFLE5 (615005)	AD	(Barcia et al., 2012, McTague et al., 2018, Heron et al., 2012)
ST3GAL3 (606494)	EIEE 15 (615006)	AR	(Edvardson et al., 2013)
TBC1D24 (613577)	EIEE 16 (615338)	AR	(Duru et al., 2010, Milh et al., 2013, Balestrini et al., 2016, Ngoh et al., 2017)
GNAO1 (139311)	EIEE 17 (615473)	AD	(Nakamura et al., 2013, Saitsu et al., 2016, Danti et al., 2017, Schorling et al., 2017)
SZT2 (615463)	EIEE 18 (615476)	AR	(Pizzino et al., 2018, Basel-Vanagaite et al., 2013)
GABRA1 (137160)	EIEE 19 (615744)	AD	(Carvill et al., 2014, Farnaes et al., 2017, Johannesen et al., 2016)
PIGA (311770)	EIEE 20 (300868)	XLR	(Belet et al., 2014, Kato et al., 2014)
NECAP1 (611623)	EIEE 21 (615833)	AR	(Alazami et al., 2014)
SLC35A2 (314375)	EIEE 22 (300896)	XLD/SM	(Ng et al., 2013, Kodera et al., 2013)
DOCK7 (615730)	EIEE 23 (615859)	AR	(Perrault et al., 2014)
HCN1 (602780)	EIEE 24 (615871)	AD	(Nava et al., 2014, Poduri, 2014, Steel et al., 2017)

Gene (MIM number)	Phenotype (MIM number)	Mode of inheritance	References
SLC13A5 (608305)	EIEE 25 (615905)	AR	(Thevenon et al., 2014, Hardies et al., 2015)
KCNB1 (600397)	EIEE 26 (616056)	AD	(Torkamani et al., 2014)
GRIN2B (138252)	EIEE 27 (616139)	AD	(Lemke et al., 2014, Platzer et al., 2017)
WWOX (605131)	EIEE 28 (616211)	AR	(Abdel-Salam et al., 2014, Tarta-Arsene et al., 2017)
AARS (601065)	EIEE 29 (616339)	AR	(Simons et al., 2015)
SIK1 (605705)	EIEE 30 (616341)	AD	(Hansen et al., 2015)
DNM1(602377)	EIEE 31 (616346)	AD	(EuroEPINOMICS-RES Consortium et al., 2014, The Deciphering Developmental Disorders Study, 2015, von Spiczak et al., 2017)
KCNA2 (176262)	EIEE 32 (616366)	AD	(Pena and Coimbra, 2015, Syrbe et al., 2015, Masnada et al., 2017, Steel et al., 2017, Hundallah et al., 2016, Corbett et al., 2016)
EEF1A2 (602959)	EIEE 33 (616409)	AD	(de Ligt et al., 2012, Veeramah et al., 2013, Lam et al., 2016)
SLC12A5 (606726)	EIEE 34 (616645)	AR	(Stodberg et al., 2015, Saito and Ishii, 2017)
ITPA (147520)	EIEE 35 (616647)	AR	(Kevelam et al., 2015)
*ALG13 (*300776)	EIEE 36 (300884),WS, LGS	XLD	(de Ligt et al., 2012, Epi4K Consortium et al., 2013, Michaud et al., 2014)
FRRS1L (604574)	EIEE 37 (616981)	AR	(Madeo et al., 2016, Shaheen et al., 2016)
ARV1 (611647)	EIEE 38 (617020)	AR	(Alazami et al., 2015, Palmer et al., 2016)
SLC25A12 (603667)	EIEE 39 (612949)	AR	(Wibom et al., 2009, Falk et al., 2014)
GUF1 (617064)	EIEE 40 (617065)	AR	(Alfaiz et al., 2016)
SLC1A2 (600300)	EIEE 41 (617105)	AD	(Epi4K Consortium, 2016, Guella et al., 2017)
CACNA1A (601011)	EIEE 42 (617106)	AD	(Epi4K Consortium et al., 2013, Epi4K Consortium, 2016)
GABRB3 (137192)	EIEE 43 (617113)	AD	(Epi4K Consortium et al., 2013, Le et al., 2017, Moller et al., 2017, Papandreou et al., 2016)
UBA5 (610552)	EIEE 44 (617132)	AR	(Arnadottir et al., 2017, Colin et al., 2016)
GABRB1 (137190)	EIEE 45 (617153)	AD	(Epi4K Consortium et al., 2013, Lien et al., 2016)
GRIN2D (602717)	EIEE 46 (617162)	AD	(Li et al., 2016)
FHF1 (601513)	EIEE 47 (617166)	AD	(Siekierska et al., 2016, Al-Mehmadi et al., 2016, Guella et al., 2016, Villeneuve et al., 2017, Takeguchi et al., 2018)
AP3B2 (602166)	EIEE 48 (617276)	AR	(Assoum et al., 2016)
DENND5A (617278)	EIEE 49 (617281)	AR	(Han et al., 2016, Anazi et al., 2017)
CAD (114010)	EIEE 50 (616457)	AR	(Ng et al., 2015, Koch et al., 2017)
MDH2 (154100)	EIEE 51 (617339)	AR	(Ait-El-Mkadem et al., 2017)
SCN1B (600235)	EIEE 52 (617350)	AR	(Patino et al., 2009, Ogiwara et al., 2012)

Gene (MIM number)	Phenotype (MIM number)	Mode of inheritance	References
SYNJ1 (604297)	EIEE 53 (617389)	AR	(Hardies et al., 2016)
HNRNPU (602869)	EIEE 54 (617391)	AD	(de Kovel et al., 2016, Hamdan et al., 2014)
PIGP (605938)	EIEE 55 (617599)	AR	(Johnstone et al., 2017)
YWHAG (605356)	EIEE 56 (617665)	AD	(Guella et al., 2017, The Deciphering Developmental Disorders Study, 2017)
KCNT2 (610044)	EIEE 57 (617771)	AD	(Gururaj et al., 2017)
NTRK2 (600456)	EIEE 58 (617830)	AD	(Hamdan et al., 2017)
GABBR2 (607340)	EIEE 59 (617904)	AD	(EuroEPINOMICS-RES Consortium et al., 2014, Hamdan et al., 2017)
CNPY3 (610774)	EIEE 60 (617929)	AR	(Mutoh et al., 2018)
ADAM22 (603709)	EIEE 61 (617933)	AR	(Muona et al., 2016)
SCN3A (182391)	EIEE 62 (617938)	AD	(Zaman et al., 2018)
CPLX1 (605032)	EIEE 63 (617976)	AR	(Redler et al., 2017, Karaca et al., 2015)
RHOBTB2 (607352)	EIEE 64 (618004)	AD	(Straub et al., 2018)
CYFIP2 (606323)	EIEE 65 (618008)	AD	(Nakashima et al., 2018)
PACS2 (610423)	EIEE 66 (618067)	AD	(Olson et al., 2018)
ATP6AP2 (300556)	ID and epilepsy	XLR	(Ramser et al., 2005)
BRAT1 (614506)	Severe EIEE (614498)	AR	(Straussberg et al., 2015, Puffenberger et al., 2012, Saunders et al., 2012, Saitsu et al., 2014b)
CACNB4 (601949)	JME (607682)	AD	(Delgado-Escueta et al., 2013)
CHRNA2 (118502)	ADNFLE (610353)	AD	(Conti et al., 2015)
CHRNA4 (118504)	ADNFLE (600513)	AD	(Wang et al., 2014)
CHRNB2 (118507)	ADNFLE (605375)	AD	(Becchetti et al., 2015)
CSTB (601145)	PME (254800)	AR	(Assenza et al., 2017)
EFHC1 (608815)	JAE/JME	AD	(Medina et al., 2008, Stogmann et al., 2006)
EPM2A (607566)	PME (254780)	AR	(Minassian et al., 1998)
GABRD (137163)	GEFS, JME	AD	(Dibbens et al., 2004)
GABRG2 (137164)	GEFS(611277) DS	AD	(Carvill et al., 2013a, Zou et al., 2017, Reinthaler et al., 2015)
GOSR2 (604027)	PME (614018)	AR	(Corbett et al., 2011)
GPHN (603930)	DEE/ DS	AD	(Dejanovic et al., 2015)
GRIN1 (138249)	DEE, HK (614254; 617820)	AD /AR	(Hamdan et al., 2011, Ohba et al., 2015, Lemke et al., 2016, Chen et al., 2017a, Zehavi et al., 2017)
KCNA1 (176260)	DEE	AD	(Rogers et al., 2018)
KCNC1 (176258)	PME (616187)	AD	(Muona et al., 2015)
KCNMA1 (600150)	DEE	AD	(Li et al., 2018)

Gene (MIM number)	Phenotype (MIM number)	Mode of inheritance	References
KCTD7 (611725)	PME (611726)	AR	(Kousi et al., 2012)
LGI1 (604619)	TLE (600512)	AD	(Boillot et al., 2014)
NHLRC1 (608072)	PME (254780)	AR	(Chan et al., 2003)
SLC1A4 (600229)	IS, non-specific DEE	AR	(Conroy et al., 2016)
IQSEC2 (300522)	DEE; IS, LGS	XLD	(Zerem et al., 2016, Zipper et al., 2017, Epi4K Consortium, 2016, Epi4K Consortium et al., 2013)
SYNGAP1 (603384)	Non-specific DEE	AD	(Mignot et al., 2016, von Stulpnagel et al., 2015)
CHD2 (602119)	Childhood onset DEE (615369)	AD	(Rauch et al., 2012, Carvill et al., 2013a, Suls et al., 2013, Lund et al., 2014)
FOXG1 (164874)	LGS, non-specific DEE	AD	(Lindy et al., 2018, Seltzer et al., 2014, Ma et al., 2016)
NEXMIF (300524)	ID, autism, epilepsy	XLD	(Lambert et al., 2018)
PLPBP (604436)	Vit B6 dependent epilepsy	AR	(Tremino and Forcada-Nadal, 2018)
ATP1A3 (182350)	Early onset DEE	AD	(Marzin et al., 2018, Qu et al., 2015)
ATP6V1A (607027)	DEE (618012)	AD	(Fassio et al., 2018)
ATRX (300032)	ID, epilepsy	XLD	(Guerrini et al., 2000)
CLTC (118955)	ID, epilepsy	AD	(Hamdan et al., 2017)
CACNA1D (114206)	Epilepsy, ASD	AD	(Pinggera et al., 2017)
CNTNAP2 (604569)	Epilepsy, ID, FCD, ASD	AR	(Smogavec et al., 2016)
DYRK1A (600855)	ID, epilepsy, microcephaly	AD	(Courcet et al., 2012, Ji et al., 2015)
GPAA1 (603048)	Epilepsy, GDD, hypotonia	AR	(Nguyen et al., 2017)
KCNJ10 (602208)	Epilepsy, ASD, EAST	AD/AR	(Sicca et al., 2016, Abdelhadi et al., 2016)
MAPK10 (602897)	Epilepsy, ID	AD	(Kunde et al., 2013)
MECP2 (300005)	Rett syndrome, epilepsy, ID	XLD/XLR	(Lindy et al., 2018)
MEF2C (600662)	Non-specific EIEE, WS	AD	(Vrecar et al., 2017, Paciorkowski et al., 2013, Bienvenu et al., 2013)
MBD5 (611472)	Epilepsy, ID, SLI, ASD	AD	(Han et al., 2017, Talkowski et al., 2011)
NACC1 (610672)	DEE, cataracts, ID (617393)	AD	(Schoch et al., 2017)
NRXN1 (600565)	PHLS (614325)/ ASD	AR/AD	(Harrison et al., 2011, Dabell et al., 2013)
KCNQ3 (602232)	Non-specific DEE, BNE	AD	(Kothur et al., 2018, Miceli et al., 1993)
PCDH12 (605622)	DEE, spasticity, microcephaly	AR	(Suzuki-Muromoto et al., 2018)
PRICKLE1 (608500)	PME (612437)	AR/AD	(Tao et al., 2011)
PRICKLE 2 (608501)	PME	AR/AD	(Tao et al., 2011)
PRRT2 (614386)	BIE, FE	AD	(Tsai et al., 2018, Kothur et al., 2018)

Gene (MIM number)	Phenotype (MIM number)	Mode of inheritance	References
PURA (60047)	LGS, non-specific DEE	AD	(Lee et al., 2018, Reijnders et al., 2018, Hunt et al., 2014)
QARS (603727)	Non-specific DEE	AR	(Kodera et al., 2015)
RYR3 (180903)	DEE	AD	(EuroEPINOMICS-RES Consortium et al., 2014)
SCARB2 (602257)	PME (254900)	AR	(Balreira et al., 2008)
SCN9A (603415)	GEFS (613863)	AD	(Singh et al., 2009)
SETD5 (615743)	Non-specific DEE	AD	(Kobayashi et al., 2016)
SLC2A1 (138140)	Generalized epilepsy	AD	(Mullen and Berkovic, 2018)
SLC6A1 (137165)	MAE, ASD, SLI	AD	(Carvill et al., 2015)
STX1B (601485)	GEFS, LKS (in 1 patient)	AD	(Vlaskamp et al., 2016, Conroy et al., 2014, Schubert et al., 2014)
TBL1XR1 (608628)	West syndrome, ASD	AD	(Saitsu et al., 2014a)
TCF4 (602272)	Pitt Hopkins syndrome	AD	(Dean, 2012)
UBE2A (312180)	ID, SI, epilepsy	XLR	(Bruinsma et al., 2016)
UBE3A (601623)	Angelman syndrome	AD	(Sadhwani et al., 2018, Kothur et al., 2018)
WDR45 (300526)	DEE	XLD	(Carvill et al., 2018)
WDR45B (609226)	ID, epilepsy, SQ	AR	(Suleiman et al., 2018)
CACNA2D1 (114204)	ID, DEE, CECTS, WS, ASD	AD	(Vergult et al., 2015, Addis et al., 2018, Iossifov et al., 2014)
PRRT2 (614386)	Infantile convulsions, PKD, LKS	AD	(Ebrahimi-Fakhari et al., 2015, Conroy et al., 2014)
RELN (600514)	Familial temporal lobe epilepsy; LKS	AD	(Dazzo et al., 2015, Conroy et al., 2014)
SMC1A (300040)	IS, FE EIMFS	XLD	(Jansen et al., 2016, Symonds et al., 2017, Huisman et al., 2017, Gorman et al., 2017)
DEPDC5 (614191)	Familial FE with variable foci, CECTS	AD	(Anderson, 2018, Addis et al., 2018)
NPRL3 (600928)	Familial FE with variable foci	AD	(Krenn et al., 2020)
ADGRV1 (602851)	LGS, JME, EOAE, GE +/- ID	AD	(Myers et al., 2018)

AD: autosomal dominant, ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy, ASD: autistic spectrum disorder, AR: autosomal recessive, BIE: benign infantile epilepsy, BNE: benign neonatal epilepsy, CECTS: childhood epilepsy with centrotemporal spikes; DEE: Developmental epileptic encephalopathy; DS: Dravet syndrome; EIEE: Early infantile epileptic encephalopathy, EAST: epilepsy, ataxia, sensorineural deafness, tubulopathy, EIMFS: Epilepsy of infancy with migrating focal seizures; EOAE: Early onset absence epilepsy; FCD: focal cortical dysplasia; FE: focal epilepsy; GEFS: generalized epilepsy with febrile seizures, GE: generalised epilepsy; ID: intellectual disability, IS: infantile spasms; JAE: juvenile absence epilepsy, JME: juvenile myoclonic epilepsy; LGS: Lennox-Gastaut syndrome; MAE: myoclonic astatic epilepsy; PME: progressive myoclonic epilepsy; NFLE: nocturnal frontal lobe epilepsy; PHLS: Pitt-Hopkins Like syndrome; PKD: paroxysmal kinesogenic dyskinesia; SLI: speech impairment; SQ: spastic quadriplegia; TLE: temporal lobe epilepsy; WS: West syndrome; XLD: X-linked dominant, XLR: X-linked recessive

Table 5-2 Qiagen Ingenuity Variant Analysis Pre-determined Filters

Filters	Notes
Confidence Filters: Include only:-	
(i) Call quality ≥ 20 in any case and any control (Phred quality scores)	Phred quality scores reflect the probability that a certain variant/base was called in error. The Phred score (Q) is logarithmically related to the probability a base was called in error ($Q=-10\log_{10}P$). A Phred score of 20 indicates that the probability of an incorrect variant call is 1/100
(ii) Read depth ≥ 10 in any case and any control	The number of reads mapped to a particular site for a given sample
(iii) Allele fraction (AF) ≥ 30 in any case and any control	The percentage of reads with the variant allele. For a well- sequenced sample with a heterozygous variant, AF should be close to 50
(iv) Outside top 5% of most exonically variable 100 bases window	The estimated nucleotide diversity (published by the 1000 Genomes project) refers to the proportion of sites at which 2 randomly chosen copies of a chromosome differ in exons of healthy people. This filter excludes variants called within 100base windows that exceed the specified percentile in the distribution of estimated nucleotide diversity.
Common Variants Filters: unless an established pathogenic variant, exclude:-	
(i) Variants that are present in 1000G with an allele frequency of > 0.1%	1000 Genomes Project (1000G): Database of variants identified during low and high coverage genomic and targeted sequencing of 26 different populations. Some cohorts contain related persons.
(ii) Variants that are present in ExAC with an allele frequency of > 0.1%	Exome Aggregation Consortium (ExAC): Database of variants identified through whole exome sequencing of 61,486 unrelated individuals as part of different disease specific and population genetic studies. Individuals with paediatric disease and their relatives were excluded.
(iii) Variants that are present in gnoMAD with an allele frequency of > 0.1%	Genome Aggregation Database (gnomAD): database of variants identified from 25,748 exomes and 15,708 genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies, totalling 141,456 individuals. Individuals with severe paediatric disorders and their first-degree relatives are excluded.
(iv) Variants that are present in ESP with an allele frequency of > 0.1%	Exome Server Project/Exome Variant Server(ESP):database of variants identified from whole exome sequencing of several large cohorts of people of European and African American ancestry.
Common Variants Filters: do not exclude:-	
(i) Variants that are present in dbSNP	dbSNP is a database of single nucleotide substitutions and short deletion/insertion polymorphisms established by NCBI in collaboration with the NHGRI. A reference (rs) is assigned to each submission. There is no stipulation regarding population MAF for variant submission.
Predicted deleterious filters: keep only variants that are:	
(i) no more than 20 bases into the intron, or	Deeply intronic variants are excluded
(ii) Pathogenic/Likely pathogenic according to ACMG, or	A set of guidelines and criteria to help classify variants (see **Figure 5-2** and **Table 5-4**)
(iii) Listed in HGMD, or	A database that collates all published gene variants responsible for inherited human disorders available at: http://www.hgmd.cf.ac.uk
(iv) predicted to lead to loss of gene function or alteration of gene function	(i) frameshift, in-frame indel, or start/stop codon change (ii) missense variant, predicted to be potentially deleterious by having CADD score > 20 (discussed in **Table 5-3**) (iii) splice site loss up to 10 bases into intron or as predicted by MaxEnt Scan* (discussed in **Table 5-3**)

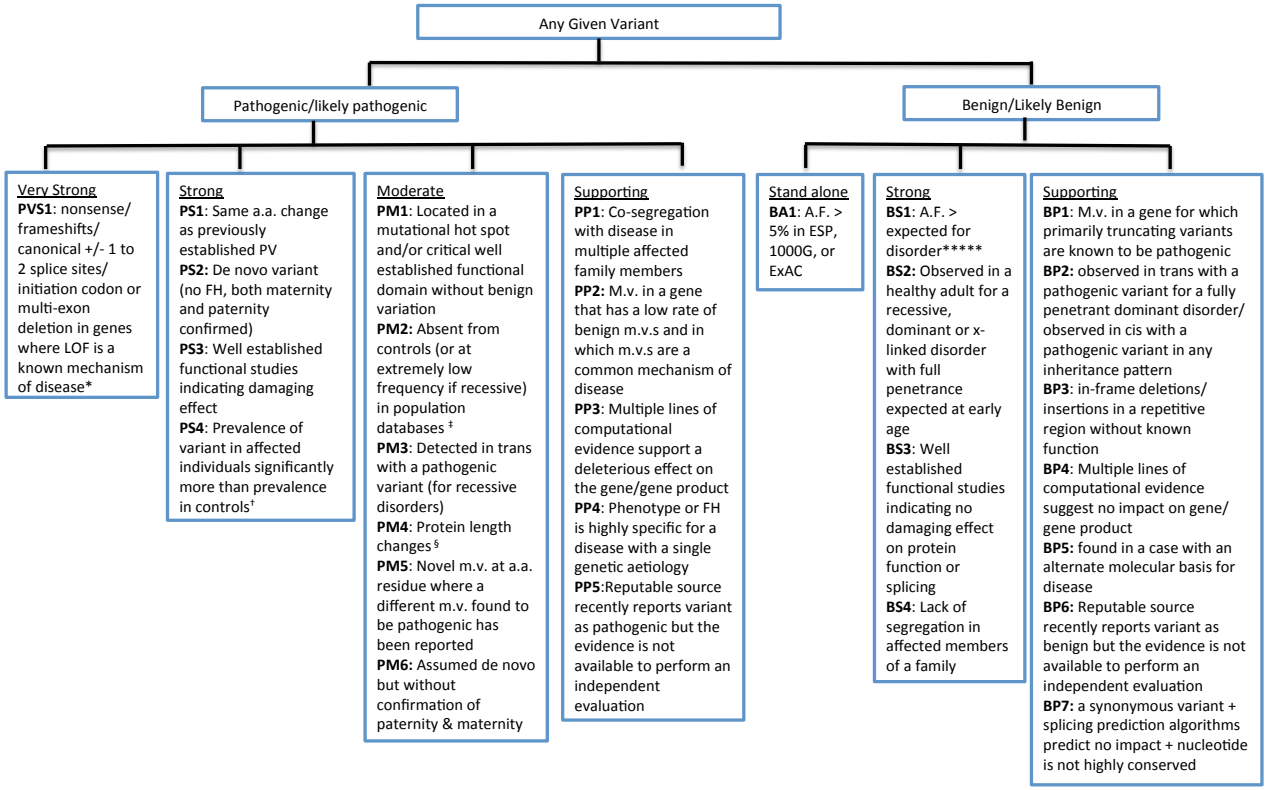
ACMG: American College of Medical Genetics, HGMD: Human Genetic Mutation database, NCBI: National Centre for Biotechnology Information; NHGRI: National Human Genome Research Institute

Table 5-3 In Silico Prediction Algorithms

In silico prediction algorithm	Description	References
CADD (combined annotation dependent depletion) https://cadd.gs.washington.edu/snv Score provided by QIVA platform	An annotation tool that was developed based on machine-learning from a training set comparing tolerated human genetic variation to simulated genetic data that represent possible de novo mutations in humans that could be present, but were not fixed in the human population. A scaled CADD score of 20 or above indicates that a variant is amongst the top 1% of predicted deleterious variants in the human genome	(Kircher et al., 2014)
SIFT (Sorting Intolerant From Tolerant) Score derived from	Uses sequence homology and physical properties of amino acids (a.a.) to predict if an a.a substitution affects protein function. A variant with a cut off score of < -2.50 is deemed deleterious	(Ng and Henikoff, 2003, Sim et al., 2012)
PROVEAN (Protein Variant Effect Analyser) version 1.1 Score derived from *	An alignment-based score that measures the change in sequence similarity of a given sequence to a protein sequence homolog before and after the introduction of a single or multiple a.a variations. A variant with a cut off score of <0.05 is predicted to be damaging.	(Choi et al., 2012)
PolyPhen-2 (Polymorphism Phenotyping version 2) Score derived through Alamut software†	A machine-learning algorithm trained on 2 datasets to predict the pathogenicity of an a.a substitution based on a number of features, including the site affected, a.a. conservation, protein homology, and a.a functional contacts. HumDiv (HD) evaluates all damaging alleles with known effects on molecular function in human mendelian disease and the differences between human proteins and closely related mammalian homologs. HumVar (HV) evaluates all human disease-causing mutations along with common human SNPs without known pathogenicity.	(Adzhubei et al., 2013)
Align GVGD (Grantham Variation Grantham Deviation) Score derived through Alamut software†	Predicts the pathogenicity of variation in the query sequence based on Grantham Variation (the amount of observed biochemical evolutionary variation at a given position in an alignment), and Grantham Deviation (the biochemical difference between the reference and substituted a.a.). The predicted effect is classified as C0, C15, C35, C45, C55 or C65, in ascending order of likelihood of pathogenicity.	(Mathe et al., 2006, Hicks et al., 2011)
Mutation Taster 2 Score derived through Alamut software†	Predicts the disease potential of a gene variant using a Bayes classifier, after studying the frequencies of single features for known disease mutations from HGMD Professional and frequencies of single features of polymorphisms from the 1000 Genomes Project. A probability value close to "1" denotes high confidence.	(Schwarz et al., 2014)
NNSplice (Neural Network Splice) Score derived through Alamut software†	A machine learning artificial neural network trained to classify splice sites based on examples of consensus splice sites and strongly correlated neighbouring positions.	(Reese et al., 1997) (Jian et al., 2014)
MaxEntScan Score derived through Alamut software†	Given a set of low-order marginal constraints estimated from available data including dependencies between neighbouring and non- neighbouring positions, this method uses the maximum entropy distribution to model splice site sequences.	(Yeo and Burge, 2004)
Splice Site Finder-like (SSF) Score derived through Alamut software†	A splice site prediction method based on position weight matrices. This involves deriving the relative frequencies of each nucleotide for a specific window around a splice site and ranking a sequence using appropriate weights for each nucleotide position.	(Shapiro and Senapathy, 1987)

* http://provean.jcvi.org; † (https://www.interactive-biosoftware.com); a.a.: amino acid; HGMD: human genome mutation database

Figure 5-2 American College of Genetics and Genomics guidelines for the interpretation of sequence variants adapted from (Richards et al., 2015)



The ACMG guidelines define characteristics that suggest any given genetic variant may more likely to be pathogenic or benign according to the algorithm above. These characteristics can then be combined using the rules described in Table 5-4 to classify a sequence variant as pathogenic, likely pathogenic, likely benign, benign or of uncertain significance. AF: allele frequency; a.a: Amino acid; FH: family history, ESP: exome sequencing project; Exac: exome aggregate consortium; 1000G: 1000 genomes project; LOF: loss of function, m.v.: missense variant *Caution with genes where LOF is not a disease causing mechanism, towards extreme 3' end of gene, splicing variants which lead to exon skipping but rest of protein left intact, or in the presence of multiple transcripts; †Odds ratio > 5.0 and confidence interval of odds ratio does not include 1.0, or observation of variant in multiple unrelated patients with the same phenotype; ‡ESP, Exac or 1000G; §In frame deletions/insertions in non-repeat regions or stop-loss variants

Table 5-4 ACMG Rules for classification of sequence variants

Pathogenic	(i). 1 Very strong (PVS1) AND (a) ≥ 1 strong (PS1-PS4); or (b) ≥ 2 moderate (PM1-PM6); or (c) 1 moderate (PM1-PM6) and 1 supporting (PP1-PP5); or (d) ≥ 2 supporting (PP1-PP5)
	(ii) ≥ 2 strong (PS1- PS4)
	(iii) 1 strong (PS1 – PS4); AND (a) ≥ 3 moderate (PM1-PM6); or (c) 2 moderate (PM1-PM6) AND ≥ 2 supporting (PP1-PP5); or (d) 1 moderate (PM1-PM6) AND ≥ 4 supporting (PP1-PP5)
Likely pathogenic	(i). 1 Very strong (PVS1) AND 1 moderate (PM1-PM6)
	(ii) 1 strong (PS1- PS4), AND 1 -2 moderate (PM1-PM6)
	(iii) 1 strong (PS1 – PS4); AND ≥ 2 supporting (PP1-PP5)
	(iv) ≥ 3 moderate (PM1-PM6)
	(v) 2 moderate (PM1-PM6) AND ≥ 2 supporting (PP1-PP5)
	(vi) 1 moderate (PM1-PM6) AND ≥ 4 supporting (PP1-PP5)
Benign	(i) 1 stand alone (BA1)
	(ii) ≥ 2 strong (BS1- BS4)
Likely Benign	(i) 1 strong (BS1-BS4); AND 1 supporting (BP1-BP7)
	(ii) ≥ 2 supporting (BP1-BP7)
Uncertain significance	(i) Other criteria shown above are not met
	(ii) The criteria for pathogenic and benign are contradictory

5.4.2 Results

The results of known Epilepsy gene panel analysis are presented in **Table 5-5**.

Table 5-5: Identification of variants in Epilepsy panel genes

De novo variants

Gene: *GABBR2* (EIEE59, AD)											
Patients Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
89 *de novo*	c.2359C>T p.R787C	-3.43 del	0.000 damaging	Class C65 (GV: 0.00 - GD: 179.53)	Disease causing (prob: 1	Probably damaging 1.00/0.990	Absent	Absent	Absent	34	ACMG: Pathogenic (PS2+PM1+PM2+PP2 +PP3)

Gene: *SCN1A* (EIEE6, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
63 *de novo*	c.748G>A p.V250I	-0.98 neutral	0.00 damaging	Class C25 (GV: 0.00 - GD: 28.68)	Disease causing (prob: 1)	Probably damaging 0.992/0.992	rs796052962; Absent	Absent	Absent	27	ACMG: Likely pathogenic (PS2+PM2+PP2) On ClinVar RCV000188844.2 (Likely pathogenic)

Possibly de novo (but no paternal DNA available for testing)

Gene: *RYR3* (DEE, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
64, AUM	c.10067G>T p.R3356L	-5.51 del	0.014 damaging	Class C0 (GV: 241.31 - GD: 90.63)	-	Probably damaging 0.989/possibly damaging 0.866	Absent	Absent	Absent	26.3	ACMG: Uncertain significance (PM2+PM6+PP3)

Inherited variants

***NPRL3* (Familial FE with variable foci, AD)**											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
45, AS, UF	c.767+1G>T	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	27.5	ACMG: Likely pathogenic (PVS1+PM2) Splice site predictions*
72, UF	c.965C>T p.P322L	-4.40 del	0.084 tolerated	probably damaging 0.997/0.994	Class C0 (GV: 208.40 - GD: 65.28)	Disease causing (prob: 1)	rs1222598806; Absent	Absent	Absent	22.9	ACMG: VUS (PM2+PP3)

* Predicted change at donor site 1 bps upstream: MaxEnt: -100.0%, NNSPLICE: -100.0%, SSF: -100.0%

Gene: *GRIN2D* (EIEE46, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
90, UM	c.1244T>C p.L415P	-2.65 del	0.049 damaging	Class C0 (GV: 251.61 - GD: 0.00)	Disease causing (prob: 1)	Probably/ possibly damaging 0.996/ 0.731	Absent	Absent	Absent	22.1	ACMG: VUS (PM2+PP2)

Gene: *CACNA2D1* (ID, DEE, CECTS, WS, ASD, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
8, UF	c.2930C>T; p.S977L	-3.62 del	0.006 damaging	Class C15 (GV: 78.78 - GD: 81.59)	-	Possibly damaging 0.821/0.543	Absent	Absent	Absent	27.4	ACMG: VUS (PM2+PP3)

Gene: *CHD2* (Childhood onset DEE, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
44, UM	c.4228G>T; p.D1410Y	-2.96 del	0.018 damaging	Class C15 (GV: 222.81 - GD: 112.38)	Disease causing (prob: 1)	Benign 0.340/0.226	rs775901148; absent	Allele freq: 0.0000043, 0 homozygotes	Absent	24.3	ACMG: VUS (PP2)

Gene: *SETD5* (Non-specific DEE, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
75, UF 86 APS	c.2462G>A; p.C821Y	-1.47 neutral	0.012 damaging	Class C0 (GV: 256.50 - GD: 17.22)	Disease causing (prob: 0.675)	Probably damaging 0.971/possibly damaging 0.543	rs376767548; Absent	Allele freq: 0.0001161; 0 homozygotes	EA: A=0.02% AA: A=0.00%	21.9	ACMG: VUS (PP3) ClinVar: VUS RCV000239041.1.

Gene: *GABRB1* (EIEE 45, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
64, UM	c.1232G>T; p.R411L	-3.15 del	0.093 tol	Class C0 (GV: 241.31 - GD: 90.63)	Disease causing (prob: 1)	Possibly damaging 0.911/0.592	rs867811295; Absent	Absent	Absent	21.8	ACMG: VUS (PM2+PP3)

Gene: *KCNQ3* (Non-specific DEE, BNE, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
74, UF	c.1226C>G; p.P409R	-4.45 del	0.009 damaging	Class C65 (GV: 0.00 - GD: 102.71)	Disease causing (prob: 1)	Probably damaging 1.00/0.998	rs149272208; Absent	Allele freq: 0.0000725; 0 homozygotes	EA: C=0.01% AA: C=0.00%	26.7	ACMG: VUS (PP3) ClinVar: VUS RCV000477245.2 RCV000187975.3

Gene: *MBD5* (Epilepsy, ID, SLI, ASD, AD)											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
90, UM	c.599G>A p.R200Q	0.17 neutral	0.000 damaging	Class C35 (GV: 0.00 - GD: 42.81)	Disease causing (prob: 1)	Probably damaging 1.00/0.995	rs149278000; MAF< 0.01	Allele freq: 0.0002137; 0 homozygotes	EA: A=0.01% - AA: A=0.02%	24.3	ACMG: VUS (PP3) ClinVar: VUS RCV000468202.1 RCV000180662.3

***SMC1A* (IS, FE EIMFS, XLD)**											
Patients/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
65, UM	c.1543G>C p.V515L	-2.81 del	0.00 damaging	Class C0 (GV: 222.52 - GD: 29.20)	Disease causing (prob: 1)	prob damaging 1.00/0.997	Absent	Absent	Absent	33	ACMG: VUS (PM2+PP3)

***ADGRV1* (LGS, JME, EOAE, GE +/- ID, AD)**											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
78, UF	c.6931G>A p.D2311N	-2.91 del	0.102 tolerated	Class C0 (GV: 213.16 - GD: 22.75)	Disease causing (prob: 1)	Probably damaging 1.00/1.00	rs764647159; Absent	Allele freq: 0.0000161, 0 homozygotes	Absent	33.0	ACMG: VUS (PP3)
29, UF	c.8465C>A p.P2822Q	-5.81 del	0.002 damaging	Class C0 (GV: 208.63 - GD: 66.83)	Disease causing (prob: 1)	Probably damaging 1.00/1.00	rs769289784, Absent	Allele freq: 0.0000392, 0 homozygotes	Absent	29.40	ACMG: VUS (PP3)
52, UF	c.13850G>A p.G4617E	-4.68 del	0.002 damaging	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Probably damaging 1.00/0.999	rs182984071, MAF: <0.01	Allele freq: 0.0000107, 0 homozygotes	Absent	27.80	ACMG: VUS (PP3)

AD: autosomal dominant, AS: affected sibling; ASD: autism spectrum disorder; APS: awaiting parental sequencing; BNE: benign neonatal epilepsy; CECTS: childhood epilepsy with centrotemporal spikes; DEE: developmental epileptic encephalopathy; EIMFS: epilepsy of infancy with migrating focal seizures; EOAE: early onset absence epilepsy; FE: focal epilepsy; GE: generalised epilepsy; ID: intellectual disability; IS: infantile spasms; LGS: Lennox Gastaut Syndrome; SLI: speech and language impairment; UF: inherited from unaffected father; UM: inherited from unaffected mother; WS: West Syndrome; XLD: X-linked dominant

5.4.3 Epilepsy Panel – Pathogenic/likely pathogenic variants

As per ACMG guidelines, pathogenic/likely pathogenic variants were discovered for three families (89, 63 and 45) in the genes, *GABBR2, SCN1A* and *NPRL3.*

5.4.3.1 GABBR2 as a candidate gene for LKS.

GABBR2 (Gamma-Aminobutyric Acid Type B Receptor Subunit 2) encodes subunit 2 of the gamma-amino-butyric acid Type B ($GABA_B$) receptor. GABBR2 and the other $GABA_B$ subunit GABBR1 (Gamma-Aminobutyric Acid Type B Receptor Subunit 1) both comprise an N-terminal extracellular domain, a seven-helix transmembrane domain, and a C-terminal intracellular domain. Within the C-terminal intracellular domain of both subunits, there is a coiled-coil sequence **(Figure 5-3**). GABBR2 binds with GABBR1 through their intracellular coiled-coil domains to form the heterodimeric $GABA_B$ receptor (Burmakina et al., 2014). This interaction is important for cell surface expression of GABBR1. GABBR1 is retained in the intracellular compartment after synthesis due to an endoplasmic reticulum retention signal. This retention signal is masked by coiled-coil interaction between GABBR1 and GABBR2 when these 2 subunits combine (Burmakina et al., 2014).

Unlike its fast-acting ionotropic $GABA_A$ receptor counterpart, which brings about rapid inhibitory synaptic response via chloride ion influx, $GABA_B$ receptors mediate slow synaptic inhibition through coupling with G-protein activation (Yoo et al., 2017).

The residual variation intolerance score (RVIS) ranks genes on the observed frequency of common functional genetic variation relative to genome wide expectation (Petrovski et al., 2013). *GABBR2* ranks among the top 5.08% of genes most intolerant to functional variation, with a residual variation intolerance score of -1.29. Furthermore, *GABBR2* knockout mice are known to exhibit memory impairment and spontaneous seizures (Gassmann et al., 2004).

The EuroEpinomics/Epi4K consortia first reported *GABBR2 de novo* variants (p.S695I and p.I705N) in 2 individuals with epileptic encephalopathy (EIEE-59) in 2014 (EuroEPINOMICS-RES Consortium et al., 2014). Hamdan et al reported another individual with *GABBR2* positive EIEE-59 (p.G693W) in 2017. Others have reported

GABBR2 mutations (p.A567T and p.A707T) in individuals with a neurodevelopmental disorder characterized by poor language and loss of hand skills (NPLHS)/ *MECP2*-negative Rett syndrome (Hamdan et al., 2017, Lopes et al., 2016, Yoo et al., 2017, Vuillaume et al., 2018).

Yoo et al performed functional investigations on the recurrent *GABBR2* p.A567T mutation reported in NPLHS and demonstrated that while the mutation did not affect protein synthesis or localization, mutant receptors had significantly reduced agonist-induced activity. They also demonstrated that the EIEE-59 mutations p.S695I and p.I705N further reduced agonist-induced activity. In addition, transfection of the p.A567T mutation into tadpoles resulted in abnormal swimming patterns and seizure-like behaviour (Yoo et al., 2017).

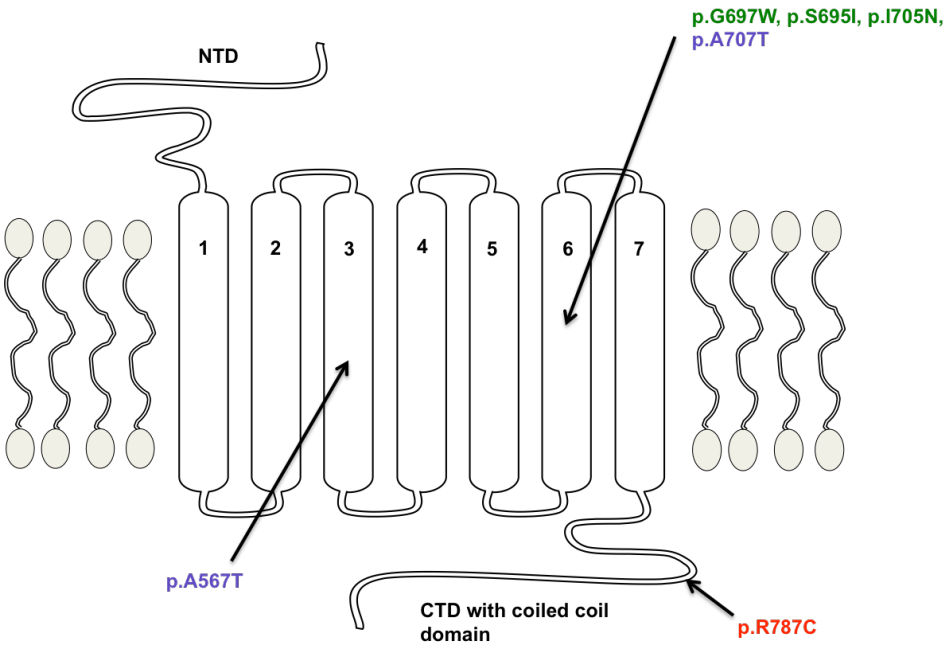
In our GOSH LKS cohort, a novel *de novo GABBR2* mutation (p.R787C) was identified in patient 89, a child with classical LKS. **Table 5-6** presents the clinical features of Patient 89 (more details in the **Appendix**), in comparison with individuals with previously reported *GABBR2* mutations. The developmental phenotypes of previously reported patients appear to be more severe than that of Patient 89. However, there are some common, overlapping clinical features including developmental regression, speech impairment, focal seizures, and autistic features.

The p.R787C is absent from ExAC, gnoMAD, 1000 genomes, ESP and dbSNP population databases. R787 is an evolutionarily well-conserved amino-acid across different species **(Figure 5-4)**. All previously reported mutations lie within different transmembrane domains (TMD) - p.A567T in TMD3 and p.S695I and p.I705N in TMD6. In contrast, R787 is predicted to lie within the coiled-coil domain **(Figure 5-3).** Mutations in this region have not previously been reported in association with disease. As discussed, the coiled-coil domain is important for interaction with GABBR1. It is possible that the substitution of the basic amino acid, arginine with hydrophobic cysteine may disrupt polar interactions within the hydrophobic core and impair cell-surface expression of GABBR1. If this is the case, this variant might have a different mechanistic effect from that of reduced agonist potency demonstrated for the other previously reported mutations; and this may account for the differences in phenotype.

All in-silico algorithms predict that this variant is likely to have a deleterious effect **(Table 5-5**). According to ACMG guidelines, this variant can thus be classified as "pathogenic" based on the presence of 1 strong (PS2), 2 moderate (PM1, PM2) and 2 supporting (PP2, PP3) criteria.

Although *GABBR2* mutations have not previously been reported in association with LKS, it is plausible that this genetic variant may be contributory to Patient 89's clinical presentation. It is therefore possible that LKS may expand the current phenotypic spectrum of *GABBR2*-related disease. The role of *GABBR2* in LKS may be further confirmed by the identification of similar patients harbouring variants in this gene and functional investigations to elucidate the effects of these variants on protein function.

Figure 5-3 Topographical domains of GABBR2 (adapted from (Hamdan et al., 2017) indicating location of identified variants in this gene



NTD: N- terminus domain, CTD: C-terminus domain. Variants in purple: Neurodevelopmental disorder with poor language and loss of hand skills (NPLHS)/Rett Syndrome mutations; Variants in green: EIEE-59 mutations; p.R787C: GABBR2 mutation identified in patient 89

Figure 5-4 Amino acid conservation data showing R787 (highlighted in red) is evolutionarily highly conserved across multiple species

H.Sapiens	744	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	793
p.troglodytes	645	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	694
C.lupus	745	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	794
B.taurus	657	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	706
M.musculus	743	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	792
R.norvegicus	743	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	792
G.gallus	693	LITLRTNPDAATQNRRFQFTQNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	742
D.rerio	739	FVTMRTNPDAATQNRRLKFTQNQKKEDSKTSTSVTSVNQANTS**R**LDGLQS	788
D.melanogaster	710	LVELKRNPQGVV-DKRVRATLRPMSKNGRRDSSVCELEQ----**R**LRDVKN	754
A.gambiae	716	LVELKRNPSGVV-DKKVRATLRPVSKN-RRDSSVCELEQ----**R**MRDVKQ	759
C.elegans	700	VIELARNPVGNEPRAYRRGLMKSVVAKTSQPMSPQPRSDSSGD**L**IGKAES	749
X.tropicalis	765	LITLRTNPDAATQNRRFQFTHNQKKEDSKTSTSVTSVNQASTS**R**LEGLQS	814

Table 5-6: Clinical features of Patient 89 in comparison with individuals with previously reported *GABBR2* mutations

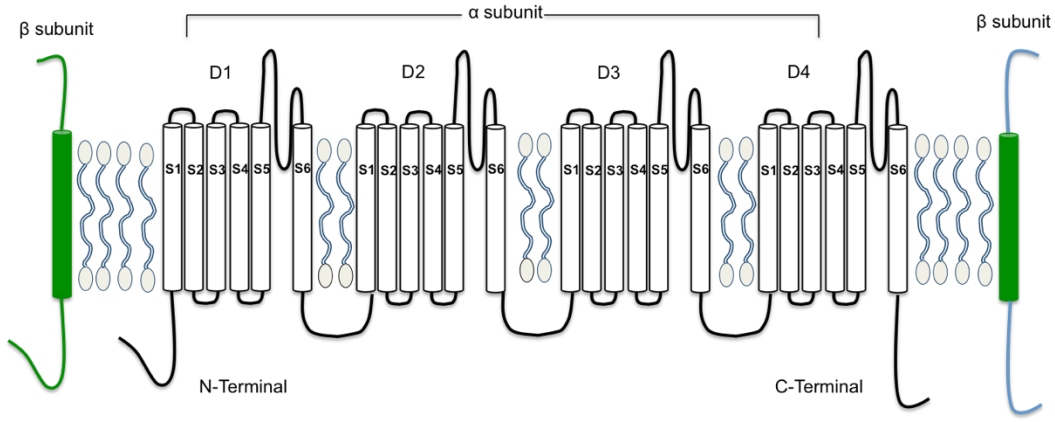
Case/Ref	Mutation effect on Protein	Diagnosis	Age of sz onset	Type of Sz	Development	Examination/other features	EEG	MRI
NLES8[1]	p.S695I	EIEE-59	1.5m	FC, T, GTCS, IS, FSIA	Profound GDD, no head control, no speech	Severe hypotonia, PEG fed	MFD, HS	N
NLES10[1]	p.I705N	EIEE-59	2.5m	IS, FSIA	N until sz onset. Severe ID, ASD, walks with support, no speech	Mild hypotonia, ataxia, DSH Severe SD	HS	N
HSJ0048[2]	p.G693W	EIEE-59	11m	IS, FSIA, GTCS	Severe ID and GDD	Hypotonia and hyporeflexia	Modified HA	Increased SAS
RTT01-1[3]	p.A567T	NPLHS/RS	9y	GTCS	DD from 6m, walked from 4y, no speech	Hand stereotypies, ataxia, Adf	NA	N
RTT02-1[3]	p.A567T	NPLHS/RS	NA	NA	DD, cannot sit/walk unsupported, no speech	Hand stereotypies, Adf, SD	N	N
RTT83-1[3]	p.A567T	NPLHS/RS	NA	NIL	DD from 4m, ID, ASD, AV, regression at 3y, lost speech and FM skills	Ataxia, DSH, SD	Slowing	N
RTT84-1[3]	p.A567T	NPLHS/RS	NA	NIL	DD from 9m, babbled at 11y, no speech	Short stature, macrocephaly, bruxism	N	NA
Pt 9[4]	p.A567T	NPLHS/RS	NA	NIL	DS then regression from 7m, severe ID, ASD, no speech	Hand stereotypies, Adf, SD	NA	NA
Pt[5]	p.A707T	NPLHS/RS	NA	NIL	Severe ID	Hand stereotypies, Adf, SD	NA	NA
Case 89	p.R787C	LKS	3y6m	GTCS, ASz, FMS	N until 3y6m, then loss of speech. Mild ID, ASD traits	Motor dyspraxia, anxiety	CTD, ESES	N

Adf: Autonomic dysfunction, ASD: autism spectrum disorder, ASz: Absence seizures, AV: abnormal vision; CTD: Centrotemporal discharges; DS: Developmental stagnation; DSH: deliberate self harm; ESES: electrical status epilepticus in slow wave sleep; FC: Focal clonic, FSIA: Focal seizures with impaired awareness, FM: fine motor; FMS: Focal motor seizures; GDD: Global developmental delay, GTCS: Generalized tonic-clonic seizures, HA: Hypsarrhythmia, IBS: Irritable bowel syndrome, IS: Infantile spasms; m: months, LKS: Landau Kleffner Syndrome, MFD: multifocal discharges; N: Normal, NA: Not available, Pt: Patient; NPLHS: Neurodevelopmental disorder with poor language and loss of hand skills; Ref: reference; RS: Rett Syndrome; SAS: subarachnoid spaces, SD: sleep disturbance, sz: seizure; y: years [1]: (EuroEPINOMICS-RES Consortium et al., 2014); [2]: (Hamdan et al., 2017)[3]: (Yoo et al., 2017); [4]: (Lopes et al., 2016); [5] (Vuillaume et al., 2018)

5.4.3.2 SCN1A as a candidate gene for LKS

SCN1A encodes the alpha subunit of a voltage-gated sodium channel found within the brain (Catterall et al., 2010). It is part of a cluster of sodium channel alpha subunit genes including *SCN2A*, *SCN3A, SCN7A* and *SCN9A* mapped to chromosome 2q24. Voltage-gated sodium channels are complexes with a large alpha subunit in association with auxiliary beta units. Alpha subunit proteins form the channel pore and have 4 homologous domains (D1-D4), each with 6 transmembrane segments (S1-S6) (**Figure 5-5**). S5 and S6 are the ion pore-lining regions. S4 in each domain acts as a voltage-sensor. Membrane depolarization is detected by this region's positively charged residues, and this leads to conformational rearrangements that open the ion pore (Catterall, 2000).

Figure 5-5: Schematic representation of SCN1A membrane topology (adapted from (Cho et al., 2018)



To date nearly 1,300 different mutations in the *SCN1A* gene have been documented in the *SCN1A* mutation database http://www.gzneurosci.com/scn1adatabase/. The epilepsy phenotypes most frequently reported with *SCN1A* mutations are those associated with febrile seizures – benign febrile seizures plus, genetic epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome (MIM 607208). However, *SCN1A* mutations have also been reported in a wide range of other epilepsy syndromes including West Syndrome, epilepsy of infancy with migrating focal seizures, Lennox-Gastaut Syndrome, juvenile absence seizures, myoclonic-atonic seizures and Panayiotopoulos syndrome (Wallace et al., 2003, Lossin, 2009, Miller and Sotero de

Menezes, 1993, Ebach et al., 2005, Carranza Rojo et al., 2011, Harkin et al., 2007). It has been estimated that approximately 1-2% of *SCN1A* mutations are associated with focal seizure epilepsy syndromes with or without febrile seizures (Lossin, 2009). In addition to pure epilepsy phenotypes, *SCN1A* variants have also been rarely reported in patients with prominent movement disorders associated with EIEE (Sadleir et al 2017) as well as neuropsychiatric phenotypes including autistic spectrum disorder and schizophrenia (Weiss et al., 2003, Papp-Hertelendi et al., 2018). Reported *SCN1A* mutations are scattered throughout the different protein domains. To date, no clear genotypic-phenotypic correlation has been established.

Importantly, there have been reports of *SCN1A* mutations in 4 patients with phenotypes within EASD. Kivity et al reported a child with a p.T875K *SCN1A* mutation childhood epilepsy with centrotemporal spikes (CECTS). This child presented with febrile seizures at the age of 1 year. These seizures resolved with age, but he re-presented at the age of 6 years with focal seizures and left centrotemporal spikes on EEG (Kivity et al., 2017). Carvill et al reported a *de novo* p.A1326V *SCN1A* mutation in an individual with epilepsy aphasia without a history of febrile seizures. No further details of the clinical phenotype are available. In the same publication, they also reported two further *SCN1A* mutations identified in individuals with epilepsy aphasia spectrum disorder: (i)a p.D45N mutation in a pair of monozygotic twins (one with epilepsy aphasia with febrile seizures, one with focal seizures), inherited from their father who has febrile seizures; and (ii) a p.R1988W mutation in a mother-daughter pair both with epilepsy aphasia and febrile seizures plus (Carvill et al., 2013a).

This study has identified a previously unreported *de novo SCN1A* mutation (p.V250I) in Patient 63 from the GOSH LKS cohort, a child with classical LKS. Patient 63 does not have a history of previous febrile seizures. She did have a generalized tonic-clonic seizure at the age of 3 months, but her parents do not recall that this was related to fever or infection. She spoke her first words at 1 year, and was joining words into sentences by the age of 2 years. At 3 years of age, she developed right focal motor seizures and her speech became unclear and difficult to understand. Her EEG showed signs of ESES. Both her language skills and EEG improved significantly on steroid therapy (further details on her case history are presented in the **Appendix**).

This identified variant has previously been submitted to ClinVar by a single submitter, without a specified phenotype and has been classified as likely pathogenic. To my knowledge it has not been previously published. It has been reported on dbSNP (rs796052962), but is absent from gnoMAD, ExAC, and 1000 genome population databases. Most *in silico* algorithms predict that this variant is likely to be pathogenic. The variant involves a highly conserved amino acid **(Figure 5-6).** It is located within S5 of the first transmembrane domain, D1, an important pore-lining region, where previously reported *SCN1A* mutations tend to cluster.

Figure 5-6: Amino acid conservation data showing V250 (highlighted in red) is evolutionarily highly conserved across multiple species

H.Sapiens	250	VMILTVFCLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEH-SIEKNITV	298
P.troglodytes	250	VMILTVFCLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEH-SIEKNITV	298
M.mulatta	250	VMILTVFCLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEH-SVEKNITV	298
C.lupus	250	VMILTVFCLSVFALIGLQLFMGNLRNKCVQWPPTNASLEEH-SIERNITV	298
B.taurus	250	VMILTVFCLSVFALIGLQLFMGNLRNKCVQWPPTNASLEEH-TVEKNITK	298
M.musculus	250	VMILTVFCLSVFALIGLQLFMGNLRNKCVQWPPTNASLEEH-SIEKNITM	298
R.norvegicus	250	VMILTVFCLSVFALIGLQLFMGNLRNKCVQWPPTNASLEEH-SIEKNVTT	298
G.gallus	251	VMILTVFCLSVFALIGLQLFMGNLRNKCLQWPPENFTLETNITSELNSTI	300

According to the ACMG guidelines, this variant would be classified as "likely pathogenic" based on the presence of 1 strong (PS2), 1 moderate (PM2) and 1 supporting (PP2) criteria. p.L263V identified in association with familial hemiplegic migraine, is the closest *SCN1A* variant to that identified in our patient with LKS. Functional investigations on p.L263V have revealed gain of function effects, with delayed fast inactivation, delayed slow inactivation, increased persistent current, and depolarizing shifts in steady-state (Kahlig et al., 2008).

Considering the available evidence, it is plausible that this variant in *SCN1A* may be contributory to Patient 63's LKS phenotype. While this is, to my knowledge, the first identification of a *SCN1A* mutation in a child with classical LKS, pathogenic and likely pathogenic *SCN1A* variants have already been identified in other cases of EASD. It is possible that EASD are within the broad phenotypic spectrum of *SCN1A*- related disorders. Further work, including functional investigations and interrogation of other EASD/LKS cohorts, will help clarify this association.

5.4.3.3 NPRL3 as a candidate gene for LKS

NPRL3 encodes nitrogen permease regulator 3 like protein. Along with NPRL2 and DEPDC5 (Dep containing protein-5), NPRL3 is part of the GATOR1 (Gap activity against Rags) complex, which is a negative regulator of mammalian target of rapamycin complex 1 (mTORC1).

The mammalian target of rapamycin (mTOR) is an ubiquitously expressed serine/threonine kinase comprised of 2 complexes, mTORC1 and mTORC2. It has important roles in the regulation of cell-growth, proliferation, cell apoptosis, autophagy, protein transcription and synthesis (Baldassari et al., 2016). Within the brain, it is involved in the regulation of neurogenesis, dendritic morphology, and long term potentiation (LTP). Disruption of the mTOR pathway has been shown to block synaptic potentiation induced by brain derived neurotrophic factor (BDNF) in hippocampal slices, and to reduce late phase LTP expression induced by high frequency stimulation. It is possible that the mTOR translational signalling pathway may regulate protein synthesis essential for LTP at synapses (Tang et al., 2002).

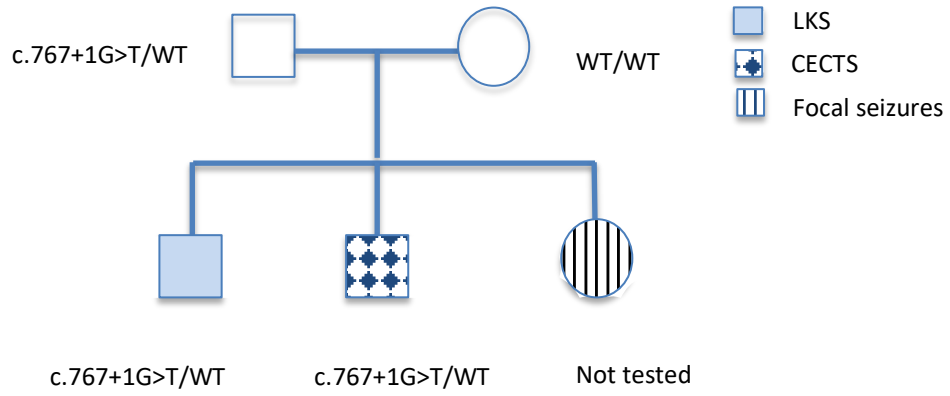
Mutations in the components of the GATOR-1 complex including loss of function variants in *NPRL3,* have been implicated in focal epilepsy syndromes with or without cortical structural abnormalities (Ricos et al., 2016). These patients have focal seizures occurring at variable foci, and this phenotype has been named familial focal epilepsy with variable foci (FFEVF). This phenotype is also associated with co-morbidities including developmental delay, speech and language impairment, autism and behavioural disorders (Baldassari et al., 2019).

It is possible that loss of function mutations in *NPRL3* lead to mTORC1 hyperactivation that results in aberrant neuronal circuit formation (Baldassari et al., 2016). It has been demonstrated that *NPRL3* knockdown in mouse neural progenitor cells leads to enhanced soma cell size, and altered filopodial outgrowth that can be reversed by the mTORC1 inhibitor rapamycin (Iffland et al., 2018).

In this study, we have identified *NPRL3* variants in 2 LKS families. The first variant, c.767+1G>T, classified as likely pathogenic by ACMG criteria, was identified in a child, Case 45, with classical LKS. He presented at the age of 5 years with right focal motor

seizures. By the age of 5 years 10 months, his speech and language skills regressed and he was non-verbal with dense auditory agnosia. He had a dramatic response to steroid therapy with return of his speech and language skills (more details in **the Appendix**). His younger brother, diagnosed with childhood epilepsy with centrotemporal spikes (CECTS) also carries the same mutation. They both have a younger sister who also has focal seizures. Their sister's DNA was not available for testing. The c.767+1G>T variant was inherited from their father. Their paternal uncle (father's brother) has a history of epilepsy, but their father has no known history of seizures (**Figure 5-7**).

Figure 5-1 Case 45 family pedigree



CECTS: Childhood epilepsy with centrotemporal spikes; LKS: Landau Kleffner Syndrome

c.767 is located at the donor splice site of intron 7 and this mutation was highly predicted to cause splice site disruption (MaxEntScan: – 100%, SSF: -100%, NNSplice: -100%) and lead to skipping of exon 7. This variant is absent from control population databases. It has been submitted to ClinVar (RCV000703657.1) by a single submitter for a proband with FFEVF, with likely pathogenic clinical significance.

Another *NPRL3* variant, p.P322L, classified as a variant of unknown significance by ACMG criteria, was identified in Case 72, a girl with classical LKS. She presented in status epilepticus associated with a viral illness at 4 years of age. After discharge, she continued having frequent seizures including focal motor seizures, generalised tonic clonic seizures, myoclonic and tonic seizures. At the age of 5 years, she had significant regression of both her expressive and receptive speech and language skills. This was associated with tantrums and aggressive behaviour. Steroid therapy improved her behaviour and speech and language skills, but had limited effect on her seizures (more

details in the **Appendix**). This proband had no significant family history for epilepsy or neurological disorders, and this variant was inherited from her unaffected father.

The p.P322L variant has been reported on dbSNP (rs1222598806), but is absent from gnoMAD, ExAC, and 1000 genome population databases. 3/5 *in silico* algorithms queried in this study predict that this variant is likely to have deleterious effects. This variant involves a highly conserved amino acid **(Figure 5-8**) and is located within the intermediary (INT) domain, which has been found to be important for interaction with NPRL2.

Both *NPRL3* variants identified in this cohort were inherited from unaffected parents. However, incomplete penetrance is well recognised for GATOR1 complex mutations (Baldassari et al., 2019). It has been proposed that a somatic "second hit" mutation mechanism may explain this phenomenon, i.e. a second somatic mutation may have occurred in a neural progenitor cell line of germline heterozygous patients, leading to compound heterozygous neural cells (Dawson et al., 2020). Another possible explanation is that, in addition to GATOR1, mTOR activity is regulated by several other non-genetic factors including insulin, growth factors, amino acids and oxidative stress. As such, phenotypic variation in *NPRL3* mutation carriers may be due to the regulation of mTOR activity by environmental factors (Baldassari et al., 2016).

Figure 5-8 Amino acid conservation data showing P322 (highlighted in red) is evolutionarily well conserved across multiple species

```
H. sapiens          293 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLAEQFSHQFPSH-DLPSVLA 341
P. troglodytes      293 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLAEQFSHQFPSH-DLPSVLA 341
M. mulatta          355 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLAEQFSHQFPSH-DLPSVLA 403
C. lupus            331 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLAEQFSRQFPAH-DLPSVLA 379
B. taurus           318 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLAEQFSRQFPSH-DLPSVLA 366
M. musculus         331 YWGKAVIIYPLCENNVYVMSPNASVCLYSPLAEQFSRQFPSH-DLPSVLA 379
R. norvegicus       318 YWGKAVIIYPLCENNVYVLSPNASVCLYSPLAEQFSRQFPSH-DLPSVLA 366
G. gallus           293 YWGKAIIIYPLCENNVYMLSPNASVCLYSPLADAFSCQFRGH-NLPSMLS 341
D. rerio            345 YWGKAIIIYPLCENNVYMLSPHANICIYSPLAEHFAVQFPGH-DLPSMLA 393
D. melanogaster     321 YWAKATIIYPLCETNVYVIAPDAPLHTKSHLVEKFSARFAGM-SLFEVIS 369
C. elegans          309 QWTRAILIYPLCNTNIY--TSATSPQPLDKMAEKFTAQFGNTIHLAAGLA 356
X. tropicalis       316 YWGKAIIIYPLCENNVYMLSPNANVGLYSSLAEQFSHQFPAH-DLPSVLS 364
```

NPRL3 mutations have not, to my knowledge, been identified in LKS. However, considering this gene' function, and the considerable phenotypic overlap between FFEVF and LKS – focal seizures, SLI, autistic traits, *NPRL3* is a very plausible candidate gene for LKS. Functional investigations and the identification of *NPRL3* variants in other

LKS cohorts will help verify if LKS may indeed be a part of the phenotypic spectrum of *NPRL3.*

5.4.4 Epilepsy Panel – Variants of Uncertain Significance

Several other variants of unknown significance were identified in known epilepsy genes **(Table 5-5).** All of these are predicted to be pathogenic by 3 or more in silico prediction algorithms. One of these (identified in *RYR3)* is not present in the proband's mother, and paternal DNA was not available; the remainder are inherited from unaffected parents. Considering the possibility of incomplete penetrance, the role these inherited variants may have to play in the pathogenesis of LKS is unclear. It is possible that even if these variants do not have a direct monogenic role in LKS causality, they may be genetic modifiers, predisposing these individuals to the phenotype.

Considerations regarding the significance of these genes to these families and LKS are presented along with the clinical features for all probands in the **Appendix**.

5.5 Panel Analysis for Genes previously associated with Epilepsy Aphasia Spectrum Disorders (EASD)

5.5.1 Methods

A panel of genes with variants previously reported in association with EASD phenotypes was assembled through literature review **(Table 5-8).** Genes within copy number variant regions identified in individuals with EASD were included, apart from those that were found to have low gene expression within the brain. Gene expression within the brain was checked using data from the Genotype Tissue Expression project (https://gtexportal.org/home).

Like with the Epilepsy gene panel analysis, all WES/WGS data was uploaded in the form of variant call files to Qiagen's Ingenuity Variant Analysis (QIVA) platform, and the same set of pre-determined filters was used to remove low confidence variants, common variants, and likely benign variants **(Table 5-2)**. The EASD gene panel was then uploaded onto the QIVA platform and the program was asked to filter for variants within these genes. The resultant variant list was exported into a Microsoft Excel spreadsheet for further manual analysis.

During manual analysis, sequence, variants that were not detected in any probands, and only found in unaffected parents were discarded.

The frequency of each variant in population databases including gnoMAD, ExAC, 1000 genomes project, ESP, and dbSNP was checked (introduced in **Table 5-3**). Higher frequency thresholds of up to 0.1% were accepted for biallelic variants, and lower thresholds of 0.05% were set for monoallelic variants. Variants occurring in higher frequencies than these set thresholds were discarded, as were identified heterozygous variants with homozygous occurrence in any control population database.

For heterozygous variants in genes that have not been established to cause disease, the likelihood of haploinsufficiency was checked using 3 methods: the HIPred score, the haploinsufficiency index (%HI) and the pLI score. These are introduced in **Table 5-7**.

Heterozygous variants in genes that were predicted not to be haploinsufficient by 2 or more of these methods were discarded. I used conservative margins for this analysis; heterozygous variants in genes that met 1 out of 3 of the following criteria: (i) pLI: > 0.50; %HI < 60%, HIPred: > 0.40, were kept.

Like with Epilepsy panel analysis, each variant's Combined Annotation Dependent Depletion (CADD) score was determined (Kircher et al., 2014) and each variant was put through five other in-silico prediction algorithms to determine the likelihood of pathogenicity. This was partly facilitated by the use of Alamut Visual software (https://www.interactive-biosoftware.com). The in-silico prediction algorithms used were described in **Table 5-3**. Variants with a CADD score of less than 20 were discarded unless they were variants affecting splice sites or frameshift variants. Only missense variants that were predicted to be pathogenic by 3 or more other different in-silico prediction algorithms were kept. Like with the epilepsy panel analysis, for variants that may be located within splice sites, splicing defect predictions from the splice site algorithms, Neural Network Splice (NNSplice), Splice Site Finder- like (SSF), and MaxEntScan (introduced in **Table 5-3**) were derived using Alamut Visual software (https://www.interactive-biosoftware.com).

Lastly, the American College of Medical Genetics and Genomics guidelines were then used to help interpret sequence variants (Richards et al., 2015). This was described in **Figure 5-2** and **Table 5-4**.

Table 5-7 Haploinsufficiency prediction methods

Haploinsufficiency prediction method	Description	References
HIPred	The HIPred method was developed from machine learning approaches integrating genomic and evolutionary information from ENSEMBL (https://www.ensembl.org), with functional annotations from the Encyclopaedia of DNA Elements consortium and the National Institute of Health (NIH) Roadmap Epigenomics Project to predict haploinsufficiency. Genes with a HIPred score of above 0.5 are predicted to be haploinsufficient.	(Shihab et al., 2017)
Haploinsufficiency index (%HI)	The %HI model uses sequence conservation, expression patterns and proximity within a gene network as predictor variables to generate predictions based on datasets of known haploinsufficient disease genes and genes disrupted by unambiguous loss-of-function variants in two or more healthy individuals. Percentages are given based on genome-wide percentiles of genes ranked according to their haploinsufficient score. 0 to 10% indicates a gene is highly likely to be haploinsufficient, whilst a score of 90-100% indicates a gene is not likely to be haploinsufficient.	(Huang et al., 2010)
pLI score	The pLI score is a score computed by the Genome Aggregation Database (gnomAD). It indicates the probability that a given gene is intolerant to heterozygous loss of function (LOF) mutations using a selection- neutral, sequence-context based mutational model to compare the observed number of rare variants in a gene to the expected number. The deviation between the observed and expected number is quantified with a Z -score. A pLI score of ≥ 0.9 indicates a gene is extremely intolerant to heterozygous LOF variation, and a pLI score of < 0.1 indicates a gene is tolerant to LOF variation.	(Samocha et al., 2014)

Table 5-8 Panel of genes associated with EASD phenotypes

Reference	Phenotype	Genes
(Lesca et al., 2012)	LKS, ECSWS	*CDH9; CDH13, CNTNAP2, DIAPH3, MDGA2, SHANK3, HSBP1*
(Conroy et al., 2014)	LKS	*BSN, EPHB2, NID2, LARP4, DIP2B, ATF1, METTL7A, SLC11A2, LETMD1, CSRNP2, TFCP2, TMEM139, PHF8, EIF3E, C7orf55, GBE1, GIPC1, SLC7A6OS; MSC, ARFGEF2, ITGB1BP1, CD59, HSPG2, COL18A1, L2HGDH, MPDZ, SLC30A3, FBXO8, BRINP3* aka *FAM5C, STX4, ZNF668, ZNF646, PRSS53, VKORC1, BCDK, KAT8, LRRTM4, PEG10, CASP2, FGD2, GPR37L,*
(Lal et al., 2013)	*CECTS*	*RBFOX1, RBFOX3*
(Panjwani et al., 2016)	CECTS	*PAX6*
(Addis et al., 2018)	CECTS	*SAMD11, NOC2L, KLHL17, TXNIP, POLR3GL, NBPF11, ARHGEF4, KCTD7, NTAN1, PDXDC1, RRN3, ARHGEF15, PTGER3, ASH1L, MSTO2P, MSTO1, YY1AP1, REG1A, CTNNA2, ERBB4, KCNIP4, GRID2, PLK2, RAB3C, PDE4D, CSPP1, ARFGEF1, NRG3, UNC13C, SMAD3, ASIC2, CCL2, C17orf102, TMEM132E, PTPRT, SLITRK2, TMEM257*
(Reinthaler et al., 2014)	CECTS, IEAD, LKS, ECSWS	*AJAP1, BIRC6, TTC27, LTBP1, SLC9A9, C3orf58, PLOD2, MUC20, TNK2, SDHAP1, TFRC, PCYT1A, TCTEX1D2, UBXN7, NCOA2, TRAM1, LACTB2, IMPA1, SLC10A5, ZFAND1, SNX16, TMEM132D, CDKN3, CNIH, GMFB, CGRRF1, SAMD4A, GCH1, WDHD1, SOCS4, MAPK1IP1L, LGALS3, FBXO34, KIAA0831, KTN1-AS1, CBLN1, ZNHIT3, PIGW, GGNBP2, DHRS11, MRM1, LHX1, AATF, ACACA, TADA2A, DUSP14, SYNRG, DDX52, RPL38, MGC16275, TTYH2, KIF19, BTBD17, GPRC5C, CD300A, RNMT, ZNF519, ANKRD30B*
Genes with more than 1 report		
(Roll et al., 2006) (Roll et al., 2010) (Lesca et al., 2012)	ECSWS	*FOXP2*
(Lesca et al., 2012) (Roll et al., 2006)	IEAD, OD	*SRPX2*
Strug et al (Lesca et al., 2012)	CECTS	*ELP4*
(Lesca et al., 2012) (Addis et al., 2018)	ECSWS/LKS CECTS	*CTNNA3*
(Conroy et al., 2014) (Addis et al., 2018)	LKS CECTS	*GRIP1*
(Conroy et al., 2014) (Reinthaler et al., 2014)	LKS CECTS/IEAD/ LKS/ECSWS	*PCDH15*
(Addis et al., 2018) (Reinthaler et al., 2014)	CECTS CECTS/IEAD/ LKS/ECSWS	*HDHD1, STS, PNPLA4*
(Lesca et al., 2012) (Addis et al., 2018)	LKS CECTS	*DLG2*

CECTS: Childhood epilepsy with centrotemporal spikes, ECSWS: Epilepsy with continuous spike waves in slow wave sleep, IEAD: Intermediate Epilepsy Aphasia Disorder; LKS: Landau Kleffner Syndrome; OD oromotor dyspraxia

5.5.2 Results

The results of EASD panel analysis are presented in ***Table 5-9***.

Table 5-9: Variants found in genes previously associated with EASD

Gene: *FOXP2* pLI:1.00, %HI:0.09, HIPred: 0.79; (ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
86, APS	c.1514C>A p.S505*	NA	NA	NA	NA	NA	Absent	Absent	Absent	41.00	ACMG: Pathogenic (PVS1 + PM2+ PM6)
75, UM	c.735G>C; p.Q245H	-3.26 del	0.004 damaging	Possibly damaging 0.828/Benign 0.371	Class C0 (GV: 42.81 - GD: 5.07)	Disease causing (prob: 1)	rs749254802 Absent	Allele freq: 0.0000109, 0 homozygotes	Absent	22	ACMG: VUS (PP3)

Gene: *CDH9* pLI: 0.99; %HI: 34.45 HIPred: 0.78 (LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
26, UM	c.1112A>C p.K371T	-4.71 del	0.001 damaging	Probably damaging 0.991/0.955	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 0.995)	rs144484970; MAF<0.01	Allele freq: 0.0003790, 0 homozygotes	EA: G=0.05% - AA: G=0.02%	26.6	AMCG: VUS (PP3)

Gene: *CTNNA3* pLI: 0.00; %HI: 2.58; HIpred: 0.47 (ECSWS, LKS, CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
34, UM	c.1532-1G>C	NA	NA	NA	NA	NA	Absent	Allele freq: 0.00000407; 0 homozygotes	Absent	34.0	ACMG: VUS (PM4) Splice site*
46, UM	c.2265+5G>A	NA	NA	NA	NA	NA	rs768745717; Absent	Allele freq: 0.00000797; 0 homozygotes	Absent	15.82	ACMG: VUS (PM4) Splice site†
48, UM	c.130C>T; p.P44S	-5.09 del	0.224 tol	Possibly damaging 0.928/0.711	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Absent	Allele freq: 0.00000407, 0 homozygotes	Absent	24.5	ACMG: VUS (PP3)

*Predicted change at acceptor site 1 bps downstream: -100.0%; MaxEnt: -100.0%; NNSPLICE: -100.0%; SSF: -100.0%;
†Predicted change at donor site 5 bps upstream: -39.6%; MaxEnt: -38.2%; NNSPLICE: -41.0%; SSF: -12.7%

Gene: *DIAPH3* pLI: 0.00; %HI: 5.85; HIPred: 0.61 (LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
73, UF	c.388A>G p.M130V	-2.58 del	0.417 tol	Possibly damaging 0.771/ Benign 0.417	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 0.999)	rs200174324; Absent	Allele freq: 0.0001176, 0 homozygotes	EA: C=0.05% AA: C=0.00%	24.2	ACMG: VUS (PP3)

Gene: *PAX6* pLI: 1.00, %HI: 0.78, HIPred: 0.87 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
89, APS	c.1304T>A p.L435*	N.A.	N.A.	N.A.	N.A.	N.A.	rs1409842258 Absent	Allele freq: 0.0000507, 0 homozygotes	Absent	38	ACMG: VUS (PP3)

Gene: *BSN* pLI: 1.00; %HI: 37.68; HIPred: 0.59 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
64, UM	c.4441T>A p.S1481T	-1.34 neut	0.012 damaging	prob damaging 0.997/0.985	Class C55 (GV: 0.00 - GD: 57.75)	Disease causing (prob: 1)	rs200382462; MAF<0.01	Allele freq: 0.0002061, 0 homozygotes	EA: A=0.01% - AA: A=0.02%	25.5	ACMG: VUS (PP3)

Gene: *EPHB2* pLI: 1.00, %HI: 2.31, HIPred: 0.78 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
64, AUM	c.3082_3083delGA p.D1028fs*13	NA	NA	NA	NA	NA	rs777486504 Absent	Allele freq: 0.0000442, 0 homozygotes	Absent	9.18	ACMG: VUS (PM6)

Gene: *DIP2B* pLI: 1.00, %HI: 27.8, HIPred: 0.64 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
86, APS	c.295C>G p.R99G	-4.44 del	0.004 damaging	Probably damaging 0.971/0.916	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Absent	Absent	Absent	26.3	ACMG: VUS (PM2+PP3)

Gene: *ARFGEF2* pLI: 1.00, %HI: 27.35, HIPred: 0.76 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
7, UF	c.742dupA p.T248fs*7	NA	NA	NA	NA	NA	Absent	Absent	Absent	25.6	ACMG: VUS (PM2)

Gene: *HSPG2* pLI: 0.00, %HI: 49.80, HIPred: 0.59 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
66, UM	c.3114delC p.I1039fs*2	NA	NA	NA	NA	NA	Absent	Absent	Absent	N.A.	ACMG: VUS (PM2)

Gene: *GRIP1* pLI: 0.92, %HI: 18.89, HIPred: 0.71 (LKS, CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
60, UM	c.446G>A p.R149Q	-1.23 neutral	0.006 damaging	Possibly damaging 0.777/ Benign 0.044	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 0.999)	rs200211966, MAF<0.01	Allele freq: 0.00004989,0 homozygous	Absent	24.2	ACMG: VUS (PP3)

Gene: *ZNF646* pLI: 0.01, %HI: 59.72, HIPred: 0.27 (LKS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
41, UF	c.1391C>T p.P464L	-6.81 del	0.022 damaging	0.999/0.990 prob damaging	Class C0 (GV: 102.71 - GD: 56.87)	Disease causing (prob: 1)	Absent	Absent	Absent	22.6	ACMG: VUS (PM2 +PP3)

Gene: *BIRC6* pLI: 1.00, %HI: 13.58, HIPred: 0.65 (CECTS, IEAD, LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
63, APS	c.8350A>G p.T2784A	-4.44 del	0.004 damaging	possibly damaging 0.956/0.899	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Absent	Absent	Absent	23.2	ACMG: VUS (PM2, PM6, PP3)

Gene: *TCTEX1D2* pLI: 0.00, %HI: 53.65, HIPred: 0.15 (CECTS, IEAD, LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
66, UM	c.329G>A p.R110H	-4.78 del	0.000 damaging	prob damaging 1.00/0.998	Class C0 (GV: 116.23 - GD: 0.00)	-	rs774457947; Absent	Allele freq: 0.00001593, 0 homozygotes	Absent	31	ACMG: VUS (PP3)

Gene: *LTBP1* pLI: 0.53, %HI: 22.20, HIPred: 0.42 (CECTS, IEAD, LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
74, UF	c.2222A>G p.H741R	-1.96 neut	0.027 damaging	prob damaging 0.971/possibly damaging 0.651	Class C0 (GV: 76.28 - GD: 28.82)	Disease causing (prob: 0.995)	rs766464179; Absent	Allele fraction: 0.00000795, 0 homozygotes	Absent	24.7	ACMG: VUS (PP3)
90, UM	c.4217G>A p.G1406D	-6.78 deleterious	0.033 damaging	prob damaging 0.998/0.952	Class C0 (GV: 206.04 - GD: 82.83)	Disease causing (prob: 1)	rs775927897; Absent	Allele fraction: 0.00000398; 0 homozygotes	Absent	28.9	ACMG: VUS (PP3)

Gene: *RBFOX1* pLI: 0.94, %HI: 0.33, HIPred: 0.75 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
84, UM	c.514C>T p.R172W	-6.67 deleterious	0.00 damaging	Prob dam 0.999/ 0.996	C65 (GV: 0.00 - GD: 101.29)	Disease causing (prob: 1)	Absent	Absent	Absent	33	ACMG: VUS (PM2 + PP3)

Gene: *RRN3* pLI:0.00, %HI:49.97, HIPred: 0.65 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
55, NPS	c.1429G>A p.G477R	-3.09 del	0.006 damaging	Probably damaging 0.997/0.960	Class C0 (GV: 90.49 - GD: 63.96)	Disease causing (prob: 1)	rs373195623; Absent	Allele freq: 0.000230, 0 homozygotes	A: T=0.00% - AA: T=0.03%	28.4	ACMG: VUS (PM6+ PP3)

Gene: *ARHGEF4* pLI:0.07, %HI:65.32, HIPred: 0.59 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
44, UM	c.1549C>T p.(Gln517*)	N.A.	N.A.	N.A.	N.A.	N.A.	rs1301518486; Absent	Absent	Absent	41	ACMG: VUS (PM2 +PM4)
30, UF	c.854G>A p.R285Q	-3.80 del	0.010 damaging	Prob damaging 1.00/1.00	Class C35 (GV: 0.00 - GD: 42.81)	Disease causing (prob: 1)	rs374920505; Absent	Allele freq: 0.00000804; 0 homozygotes	EA: A=0.01% AA: A=0.00%	33	ACMG: VUS (PP3)

Gene: *SLC9A9* pLI: 0.00, %HI: 7.02, HIPred: 0.44 (CECTS, IEAD, LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
84, UM	c.481T>A p.S161T	-1.78 neutral	0.032 damaging	prob damaging 0.992/0.987	Class C0 (GV: 209.31 - GD: 29.38)	Disease causing (prob: 1)	rs201860661; Absent	Allele freq: 0.00006022; 0 homozygotes	EA: T=0.01% - AA: T=0.00	24.7	ACMG: VUS (PP3)

Gene: *PDE4D* pLI: 0.98 , %HI: 4.23, HIPred: 0.79 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
70, UM	c.1286A>G p.Q429R	-3.28 del	0.040 damaging	possibly damaging 0.492/0.808	Class C0 (GV: 241.31 - GD: 0.00)	Disease causing (prob: 1)	rs1196836267; Absent	Allele freq: 0.000009307, 0 homozygous	Absent	32	ACMG: VUS (PP3)

Gene: *CSPP1* pLI: 0.00, %HI: 45.84, HIPred: 0.33 (CECTS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
86, APS	c.1070C>A p.S357Y	-1.86 neut	0.005 damaging	prob damaging 0.988/possibly damaging 0.686	Class C15 (GV: 103.97 - GD: 81.38)	Polymorphism (prob: 0.886)	rs374330054; absent	Allele freq: 0.0001726, 0 homozygous	EA: A=0.01% - AA: A=0.00%	22.6	ACMG: VUS (PM6+PP3)
24, UF	c.2736A>C p.R912S	-4.06 del	0.002 damaging	prob damaging 0.996/0.990	Class C0 (GV: 353.86 - GD: 0.00)	Polymorphism (prob: 0.852)	rs201720914; absent; MAF<0.01	Allele freq: 0.0001034, 0 homozygous	EA: C=0.02% - AA: C=0.05%	23	ACMG: VUS (PP3)

Gene: *LGALS3* pLI:0.00, %HI:59.56, HIPred: 0.58 (CECTS, IEAD, LKS, ECSWS)											
Patients/ Inheritance	Mutation	Provean	SIFT	Polyphen	GVGD	Mutation taster	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG Classification/Other
78, UM	c.484C>T p.R162C	-7.98 del	0.000 damaging	Prob damaging 1.00/0.995	Class C65 (GV: 0.00 - GD: 179.53)	Disease causing (prob: 1)	rs376506811; Absent	Allele freq: 0.00002848, 0 homozygous	EA: T=0.01% - AA: T=0.00%	33	ACMG: VUS (PP3)

AD: autosomal dominant, APS: awaiting parental Sanger sequencing; CECTS: childhood epilepsy with centrotemporal spikes; ECSWS: epilepsy with continuous spike waves in slow wave sleep; IEAD: intermediate epilepsy aphasia disorder; LKS: Landau Kleffner Syndrome; NPS: no parental samples; UF: inherited from unaffected father; UM: inherited from unaffected mother

5.5.3 EASD Panel – Pathogenic/Likely Pathogenic Variants

Only one pathogenic variant was identified in this analysis. This was a nonsense variant in the gene *FOXP2.*

5.5.3.1 FOXP2 as a candidate gene for LKS

FOXP2 encodes Foxhead box P protein 2, a member of a family transcription factors with important roles in developmental processes of several organ systems including pulmonary, cardiac, immune and nervous systems. *FOXP2* is highly expressed in the central nervous system and coordinates molecular pathways important for brain development and function (Co et al., 2020).

FOXP2 has been strongly associated with neurodevelopmental disorders with speech and language impairment. This was first discovered through genetic investigation of a large family pedigree with 15 members across 3 generations affected by both expressive and receptive speech and language impairment. All affected individuals carried a heterozygous missense p.R553H mutation in *FOXP2* . In addition, it was discovered that one other unrelated individual with similar symptoms had a translocation breakpoint disrupting the same gene (Lai et al., 2001). Since then, several other mutations in *FOXP2* have been identified, strengthening the case that heterozygous loss of function mutations in *FOXP2* lead to a speech and language disorder characterized by difficulties with speech motor planning and prosody of sounds, syllables and words, a phenotype subsequently named childhood apraxia of speech (CAS) (Morgan et al., 1993).

Heterozygous mice with *FOXP2* mutations have altered ultrasonic vocalisations from neonatal age to adulthood and show signs of defects in auditory-motor association and motor skill learning. It has also been demonstrated that *FOXP2* mutant mice have absence of striatal long term depression, changes in short-term plasticity and altered neurotransmitter levels- raised dopamine in the globus pallidus and nucleus accumbens, increased serotonin in the nucleus accumbens and reduced gamma-amino-butyric acid (GABA) in the frontal cortex (Co et al., 2020). Murine studies have also shown that through the regulation of the expression of the *SRPX2* (sushi-repeat containing domain protein X-linked 2) gene, *FOXP2* regulates synaptogenesis and the development of

neural circuits (Roll et al., 2010). Neuroimaging for *FOXP2* mutation human carriers has demonstrated structural and functional abnormalities in distributed brain circuits, particularly those involving the cortex, basal ganglia and cerebellum (Vargha-Khadem et al., 2005, Liégeois et al., 2003).

In addition to speech and language disorders, co-morbidities identified in humans with *FOXP2* mutations include behavioural disorders, autistic features, fine motor difficulties and mild intellectual disability. (Morgan et al., 1993).

Many studies have also investigated the possibility that *FOXP2* mutations may contribute to other disorders with speech and language difficulties such as autism, specific language impairment and schizophrenia (Estruch et al., 2016). Overall, there is no conclusive evidence yet to suggest that *FOXP2* has a causative role in these other disorders (Morgan et al., 1993). In 2010, Roll et al screened for *FOXP2* variants in a cohort of 32 patients with epilepsy aphasia spectrum disorders (EASD). They identified a p.M406T *FOXP2* variant in a girl with focal epilepsy with electrical status epilepticus in slow wave sleep (ESES), cognitive and language deficits and polymicrogyria of the left Rolandic operculum. This variant is absent from all control population databases and is predicted to be deleterious by all in-silico prediction algorithms. However, this variant did not segregate with affected status in this proband's family- her father, brother and sister also carried the variant but did not have any obvious neurological abnormalities. The incomplete penetrance of this disorder led the authors to conclude that this variant may have a contributory but not causal role to play in the aetiology of the proband's phenotype (Roll et al., 2010). In 2012, Lesca et al reported their study screening for *FOXP2* variants in 61 patients with epilepsy aphasia spectrum disorders (EASD). They discovered the same *FOXP2* variant, previously reported by Roll et al (p.M406T) in a girl with congenital right hemiplegia, left hemispheric polymicrogyria, nocturnal seizures, language regression and ESES. Additionally, they discovered a variant of uncertain significance, an in-frame deletion, p.Q191del, within the first polyglutamine tract of FOXP2, in another individual with idiopathic epilepsy syndrome with continuous spike waves in slow wave sleep (ECSWS) (Lesca et al., 2012).

Functional investigations for p.M406T have produced contradicting results. Roll et al reported that this variant led to altered nuclear localization of FOXP2, and that this

variant led to loss of repression of the *SRPX2* promoter compared to wild-type FOXP2 (Roll et al., 2010). However, a separate study group reported that this variant had no significant effect on nuclear localization of FOXP2 and had similar transcription repressor activity as wild-type FOXP2 (Estruch et al., 2016).

FOXP2 contains a long polyglutamine tract of 40 residues (p.Q152-Q191) and a shorter polyglutamine tract of 10 residues (p.Q200 to Q209). Deletions in the polyglutamine tract like p.Q191del have been described in neurodevelopmental disorders, including autism spectrum disorders (Estruch et al., 2016). However such deletions are also observed in general population databases, suggesting that they may represent risk factors or contribute to a phenotype but that they are not likely to be causal variants with high penetrance. The reported p.Q191del is present on gnomAD at an allele frequency of 0.3%. Studies investigating the effects of *FOXP2* polyglutamine tract truncation have suggested that such variants do not have significant effect on FOXP2 function (Estruch et al., 2016).

In this study, we have identified a novel p.S505* nonsense mutation in Case 86, a girl with a phenotype that seems to fall somewhere in between CAS and EASD (more details on her case history are presented in the **Appendix**). Although she was referred to the Developmental Epilepsy Clinic to evaluate the possibility of Landau Kleffner Syndrome (LKS), there was no history of speech and language regression, so her phenotype was atypical for LKS. She had significant verbal dyspraxia which can match the phenotype of CAS. However, her electroencephalogram (EEG) was abnormal with frequent focal discharges which activated in sleep, occupying up to 50% of the sleep recording. Abnormal EEG findings have not, to my knowledge, been described in *FOXP2* CAS. Most reports have not included EEG findings, but those who did have reported normal EEGs (Morgan et al., 1993). p.S505* is absent from all control population databases. It interrupts the gene's reading frame with a premature stop codon and is likely to result in mRNA nonsense mediated decay. I have not yet been able to verify the inheritance of this variant. Family 86 was one of the 10 families for whom DNA arrived late and who only had proband DNA sent for WES (**Figure 5-1)**. At the time of writing, I have not been able to Sanger sequence her parental DNA samples due to COVID-19 imposed laboratory restrictions. Nonetheless, this variant has been classified as pathogenic by ACMG criteria.

A second novel variant in *FOXP2*, p.Q245H, was detected in this cohort, in a family with 2 affected brothers (Family 75). The older brother had classical LKS characterized by speech and language regression at the age of 19 months and centro-temporal epileptiform discharges on EEG. The younger brother presented at the age of 10 years with behavioural difficulties, seizures and ESES on EEG. The younger brother did not have as prominent speech and language difficulties as his older brother (more details on case histories in the Appendix). The p.Q245H variant was identified in the younger brother and their unaffected mother. Unfortunately, the older brother's DNA was not available for testing. This variant occurs only rarely in control population databases with an allele frequency of 0.001% in gnomAD. It involves a highly conserved amino- acid (**Figure 5-9**) and is predicted to be damaging by most in-silico prediction algorithms (***Table 5-9***).

Figure 5-9 Amino acid conservation data showing Q245 (highlighted in red) is evolutionarily well conserved across multiple species

```
H. sapiens          236 AAQQLVFQQQLLQMQQLQQQQHLLSLQRQGLISIPPGQAALPVQSLPQAG 285
P. troglodytes      212 AAQQLVFQQQLLQMQQLQQQQHLLSLQRQGLISIPPGQAALPVQSLPQAG 261
C. lupus            233 AAQQLVFQQQLLQMQQLQQQQHLLSLQRQGLISIPPGQAALPVQSLPQAG 282
B. taurus           241 AAQQLVFQQQLLQMQQLQQQQHLLSLQRQGLISIPPGQAALPVQSLPQAG 290
R. norvegicus       206 AAQQLVFQQQLLQMQQLQQQQHLLSLQCQGLISIPPGQAALPVQSLPQAG 255
X. tropicalis       204 AAQQLVFQQQLLQMQQLQQQQHLLNLQRQGLISIPPSQSALPVQSLPQAG 253
```

Considering the above evidence, it is likely that the p.S505* bears high contributory or even causative clinical significance for Case 86. It is a rare, likely loss of function variant in a child whose phenotype can be consistent with CAS, apart from her EEG findings. This variant is considered alongside other genetic variants identified for this proband in the **Appendix**. The clinical significance of the p.Q245H variant for family 75 is less certain. DNA is not available for testing for the older brother, and the younger brother did not have prominent verbal dyspraxia. Additionally, this variant was inherited from an unaffected mother. Whilst Roll et al reported incomplete penetrance for the p.M406T variant for ECSWS, complete penetrance is reported for *FOXP2* variants in CAS (Morgan et al., 1993).

At present, there is little to support *FOXP2* as a causative candidate gene for LKS or EASD. The fact that case 86 had abnormal EEG findings may suggest that the phenotypic spectrum of *FOXP2* mutations may include epilepsy. However, Case 86 also has other identified genetic variants that may also account for/contribute to a seizure phenotype

(**Appendix).** Whilst *FOXP2* variants have previously been identified in individuals with EASD (Roll et al., 2010, Lesca et al., 2012), functional studies investigating these variants have been unconvincing. Considering this and the lack of segregation with affected phenotype for the p.M406T variant, it is possible that these were incidental findings. Nonetheless, the strong association of this gene with speech and language disorders, makes it difficult to discount the possibility that this gene may have, at least, a contributory role to play in LKS and EASD.

5.5.4 EASD Panel – Variants of uncertain significance

Variants of uncertain significance were identified in 21 genes from the EASD panel. Although these genes do not have well-established causative roles in epilepsy, considering that variants in these genes have already been reported in relation to other EASD individuals, it is quite likely that they may have a contributory role to play in the pathogenesis of LKS.

The majority of these genes have well- established roles in mechanisms linked to seizure manifestation and long-term potentiation (LTP) which make them attractive candidates for LKS. This is further discussed in **Chapter 6, Section 6.5.**

The function of these genes and the clinical significance of these genetic variants to the families they were discovered in, and to LKS as a phenotype, are considered in the **Appendix**, alongside a summary of each proband's clinical features and other genetic findings.

A few interesting candidates that may merit additional brief mention here include *BSN, CTNNA3* and *ARHGEF4.*

BSN is almost exclusively expressed in the brain. It encodes Bassoon, a pre-synaptic cytomatrix protein that is believed to have important roles in organizing the cytomatrix for neurotransmitter release, glutamatergic synapse maturation and neuro-plasticity (tom Dieck et al., 1998, Lanore et al., 2010). The variant identified in this study, p.S1481T has previously also been identified in another LKS individual by Conroy et al in 2014. This same study also identified another *BSN* variant in the adjacent amino-acid, p.P1482L (Conroy et al., 2014). These variants are located in exon 5, which has been

found to code for the well-conserved central portion of BSN, a domain that is vital for anchoring the protein to the cytomatrix active zone. Murine models with deletions of exons 4 and 5 develop epileptic seizures (Altrock et al., 2003).

CTNNA3 encodes Catenin Alpha-3, an actin-binding protein that is an important component of catenin-cadherin cell-cell adhesion. Evidence suggests that this protein may have a role in the stabilisation of neuronal dendritic spines in the hippocampus (Abe et al., 2004). This protein has also been shown to have important roles in maintaining neuronal cell-junctions and cell-signalling within the cerebellum (Folmsbee et al., 2016). These cerebellar functions have, in turn, been implicated in autism and in the development of speech and language skills (Hampson and Blatt, 2015).

Monoallelic copy number variants (CNV) disrupting only *CTNNA3* were identified in 4 individuals with ECSWS or LKS (Lesca et al., 2012). Addis et al also discovered a CNV involving only this gene in a child with childhood epilepsy with centrotemporal spikes (CECTS), attention-deficit-hyperactivity disorder and learning difficulties (Addis et al., 2018). Additionally, rare variants in this gene have been associated with autism spectrum disorders and Alzheimer's disease (Butler et al., 2015, Bacchelli et al., 2014, Miyashita et al., 2007). Outside neurological disorders, autosomal dominant variants in *CTNNA3* have been associated with familial arrhythmogenic right ventricular dysplasia with incomplete penetrance (van Hengel et al., 2013). This study has identified *CTNNA3* variants in 3 males with classical LKS. All these variants were inherited from unaffected mothers. Two of these variants are located within splice site regions and have been predicted to lead to splicing defects.

ARHGEF4 encodes a Rho-guanine nucleotide exchange factor that is very highly expressed in the brain. ARHGEF4 has a role in the regulation of structural plasticity of dendritic spines in the hippocampus (Oh et al., 2018). Through acting as a specific guanine nucleotide exchange factor (GEF) for the GTPase, CDC42 (cell-division cycle-42), ARHGEF4 also has a role in neuronal dendritic outgrowth and neuro-plasticity (Oh et al., 2018). *ARHGEF4* is one of 5 genes contained in a recurrent 2q12.1 CNV hotspot identified in developmental epilepsy encephalopathy with speech and language impairment, intellectual difficulties and behavioural disorders (Dharmadhikari et al., 2012). A CNV containing only this gene has also been identified in an individual with

CECTS and a family history of reading and speech difficulties (Addis et al., 2018). This study has identified *ARHGEF4* variants in 2 individuals- a protein truncating nonsense variant, p.Gln517*, that is absent from all control population databases, in a girl with classical LKS, and a rare missense variant p.R285Q in a boy with speech and language difficulties with electrical status epilepticus in slow wave sleep (ESES). p.R285 is a highly conserved amino-acid within the protein's Dbl homology domain, a domain essential for its GEF activity.

5.6 Summary

GRIN2A accounts for only 8- 20% of individuals with LKS or EASD. In the search for other candidate genes for LKS, this chapter has explored the possibility that genes with association to other epilepsy syndromes may also have LKS as part of their phenotypic spectrum. Our results suggest that LKS may be part of the phenotypic spectrum of *GABBR2*, a gene associated with early infantile epileptic encephalopathy (EIEE-59) and a neurodevelopmental disorder characterized by poor language and loss of hand skills (NPLHS). It is also likely that LKS is a part of the wide phenotypic spectrum of *SCN1A,* a gene well-established as a causative factor in febrile seizure phenotypes.

In addition, this chapter has evaluated the possibility that this cohort may augment previous genetic findings reported in other LKS and EASD studies. This part of the study has identified several interesting candidate genes. Whilst there may be insufficient evidence for a causative monogenic role for some of these candidates, considering the fact that many of these genes have important functions in long term potentiation and seizure mechanisms, and the fact that variants in these genes have been recurrently identified in LKS/EASD, it is quite possible that they may have a contributory role in the pathogenesis of these disorders.

6 Chapter 6: Molecular Genetic Investigation of *GRIN2A*-negative LKS Part 2 – Individual Triome Analyses

6.1 Individual Familial Triome Analysis

After first-pass panel analysis to identify variants in previously reported genes associated with epilepsy and/or epilepsy-aphasia spectrum, I then undertook individual familial triome analysis to identify novel putative candidate genes associated with LKS.

In the LKS cohort, I identified a total of 38 *GRIN2A*- negative familial triomes. As reported in Chapter 5, 11 triomes were sent for whole exome sequencing (WES) and 27 triomes were sent for whole genome sequencing (**Figure 5-1**).

WES/WGS data from each of the 38 *GRIN2A*-negative triomes, were analysed one family, at a time, using: (i) *de novo* dominant, (ii) autosomal recessive, and (iii) x-linked recessive models of inheritance (for male patients).

The remaining WES/WGS dataset [incomplete triomes (n=6) or DNA received at a late stage of the PhD (n=10)], was then interrogated for variants in the candidate genes identified through the triome analysis described above (**Figure 5-1).**

To allow for the possibility of incomplete penetrance, as observed in *GRIN2A*- positive EASD families, I also carried out an additional analysis to identify genes in which rare dominantly inherited variants were carried by 3 or more LKS families.

Sanger sequencing was used to verify relevant genetic variants. Some variants, identified towards the end of this study could not be verified by Sanger sequencing in time for the writing of this thesis due to COVID-19 pandemic imposed laboratory restrictions. For these variants, raw sequence .bam files and Integrated Genomics Viewer (IGV) software (http://software.broadinstitute.org/) were used to check coverage and read depth to verify the reliability of the variant call. For the 11 families whose DNA was received at a late stage of this PhD and only proband DNA were sent for WES, direct Sanger sequencing (where possible) was carried out to ascertain the inheritance of relevant variants, if parental DNA was available (**Figure 5-1**).

6.2 Familial triome *de novo* dominant analysis

6.2.1 Methods

Detailed methods for WES/WGS were described in Chapter 2.5 – 2.6.

35 triomes were analysed individually, one at a time. 3 triomes (families 3, 45 and 75) were excluded from *de novo* analysis due to their family history (**Chapter 3,Figure 3-2**).

WES/WGS data for each triome was uploaded in the form of variant call files to Qiagen's Ingenuity Variant Analysis (QIVA) platform. A set of pre-determined filters similar to the ones used for panel analysis was used to remove low confidence variants, common variants, and likely benign variants (**Table 6-1**). A genetic analysis filter was then added to sift for *de novo* variants (**Table 6-2**).

Table 6-1: QIVA Pre-determined filters

CONFIDENCE FILTERS: Include only:-
(i) Call quality ≥ 20 in any case and any control (ii) Read depth ≥ 10 in any case and any control (iii) Allele fraction ≥ 35 in any case and ≥ 10 in any control* (iv) Outside top 5% of most exonically variable 100 bases window
COMMON VARIANTS FILTERS: unless an established pathogenic variant, exclude:-
(i) Variants that are present in 1000G with an allele frequency of > 0.01% (ii) Variants that are present in ExAC with an allele frequency of > 0.01% (iii) Variants that are present in gnoMAD with an allele frequency of > 0.01% (iv) Variants that are present in ESP with an allele frequency of > 0.01%
PREDICTED DELETERIOUS FILTERS: include only variants that are:
(i) no more than 20 bases into the intron (i.e no further than 20 bases from the exon border) (ii) Pathogenic/Likely pathogenic according to ACMG guidelines (reference) (iii) either listed in Human Gene Mutation Database OR:- predicted to lead to loss of gene function or alteration of gene function:- (i) frameshift, in-frame indel, or start/stop codon change (ii) missense variant, predicted to be potentially deleterious by having CADD score > 20 (iv) splice site loss up to 10 bases into intron or as predicted by MaxEnt Scan*

Table 6-2: Genetic Analysis Filter for *de novo* variants

Use recommended settings for: **inferred gain- or loss- of- function variants*** **Restrict to de novo variants**.	
CASE (affected patient) samples. **Keep only**:-	CONTROL (parental) Samples. **Exclude:-**
Haploinsufficient	Homozygous/ Compound heterozygous
Hemizygous	Heterozygous/Het-ambiguous
Het-ambiguous	Haploinsufficient
Heterozygous	Hemizygous
AND:-	AND:-
The genotypes selected above occur in at least **1** of the 1 case samples (100%) at **variant** level	The genotypes selected above occur in at least **1** of the 2 control samples (50%) at **variant** level

*The filter is configured to identify variants that are expected to cause gain-or loss-of function at gene level, in cases vs controls

The resultant variant list for each family was exported into a Microsoft Excel spreadsheet for further manual analysis. During manual analysis, variants meeting the criteria set out in **Table 6-3** were discarded.

Table 6-3: Manual filtering for *de novo* variants

Discarded variants	**Method**
Homozygous occurrence in any control population database	Checking population databases including gnoMAD, ExAC, 1000 genomes project, ESP, and dbSNP
CADD score of < 20 unless the variant is within a splice site region or is a frame-shift variant	The utility of the CADD score was introduced in Chapter 5 (**Table 5-3**).
Predicted to be benign by in-silico prediction algorithms.	Each variant was put through five *in silico* prediction algorithms to determine the likelihood of pathogenicity (SIFT, PROVEAN, GVGD, Mutation Taster and PolyPHEN). This was partly facilitated by the use of AVS. The in-silico prediction algorithms used were introduced in Chapter 5 (**Table 5-3**). Only variants predicted to be pathogenic by 3 or more different *in silico* prediction algorithms were kept for downstream analysis
Variants in splice site regions that were not predicted to lead to splicing defects	Splicing defect predictions from the splice site algorithms, Neural Network Splice (NNSplice), Splice Site Finder- like (SSF), and MaxEntScan (introduced in **Chapter 5.4.1, Table 5 3)** were derived using AVS. A combination of a percentage difference of 15% or more for MaxEntScan and 5% or more for NNSplice and SSF was taken to imply a likely splice site disruption.
Variants in genes that were predicted to tolerate haploinsufficiency	3 methods were used to predict haploinsufficiency: the HIPred score, the haploinsufficiency index (%HI) and the pLI score. These were introduced in **Table 5-7**. Heterozygous variants in genes that met at least 1 out of 3 of the following criteria: (i) pLI: > 0.50; %HI < 60, HIPred: > 0.40, were kept.
Genes not expressed in the brain	Gene expression within the brain was checked using data from the UK Brain Expression Consortium (http://www.braineac.org) and data from the Genotype Tissue Expression project (https://gtexportal.org/home)

AVS: Alamut Visual software (https://www.interactive-biosoftware.com).

The American College of Medical Genetics and Genomics (ACMG) guidelines were used to aid interpretation the remaining sequence variants (Richards et al., 2015). This was described in **Figure 5-2** and **Table 5-4**.

As mentioned above, the entire LKS cohort dataset was interrogated for variants in all identified potential candidate genes from this *de novo* analysis. To allow for incomplete penetrance, inherited autosomal dominant variants in candidate genes were also considered.

6.2.2 Results

The results for familial triome *de novo* analysis are presented in **Table 6-4**.

Table 6-4: Candidate genes identified from *de novo* analysis

Pathogenic/Likely pathogenic variants

Gene: *TRPC1* pLI: 0.78, %HI:16.97, HIPred: 0.73											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
46, *de novo*	c.961-2A>C	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	24.0	ACMG: Likely Pathogenic (PS2+PM2) Splicing prediction*

*Predicted change at acceptor site 2 bps downstream: MaxEnt: -100.0%; NNSPLICE: -100.0%; SSF: -100.0%

Gene: *WDFY3* pLI: 1.00, %HI:21.08, HIPred: 0.76											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
30, *de novo*	c.2866delG; p.D956fs*5	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	-	ACMG: Pathogenic (PVS1+PS2+PM2)

Gene: *ERRFI1* pLI: 0.05, %HI: 74.63, HIPRed: 0.80											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
53, *de novo*	c.566_567delCT; p.S189*	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	-	ACMG: Likely pathogenic (PS2+PM2+PM4)

Gene: *RORB* pLI:1.00, %HI: 2.67, HIPred: 0.82											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
57, *de novo*	c.896G>A; p.C299Y	-4.75 del	0.002 damaging	prob damaging 0.999/0.982	Class C0 (GV: 199.60 - GD:82.07)	Disease causing (prob: 1)	Absent	Absent	Absent	27.9	ACMG: Likely pathogenic (PS2+PM2+PP3)

Gene: *CTXN3* pLI: 0.54, %HI: 52.57, HIPRed: 0.36											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
48, *de novo*	c.164C>T; p.P55L	-10.00 del	0.00 damaging	Class C65 (GV: 0.00 - GD: 97.78)	Disease causing (prob: 1)	Probably damaging 1.00/0.999	Absent	Absent	Absent	29.3	ACMG: Likely pathogenic (PS2+PM2+PP3)

Gene: *KMT2A* pLI:1.00, %HI: 17.00, HIPred: 0.73											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
58, *de novo*	c.3460C>T; p.R1154W	-6.02 del	0.000 damaging	Prob damaging 1.00	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Absent	Absent	Absent	35	ACMG: Likely pathogenic (PS2+PM2+PP3)

Gene: *SOCS7* pLI:1.00, %HI: 27.84, HIPred: 0.66											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
43, *de novo*	c.1609G>C; p.D537H	-1.15 neut	0.018 damaging	Class C0 (GV: 353.86 - GD: 0.00)	-	Probably Damaging 0.990, possibly damaging 0.852	Absent	Absent	Absent	24.9	ACMG: Likely pathogenic (PS2+PM2+PP3)

Gene: *RBM15* pLI: 1.00, %HI: 13.63, HIPred: 0.66											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
81, de novo	c.1177delG; p.A393fs*11	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	-	ACMG: Likely Pathogenic (PS2+PM2)

Gene: *NLRP3* pLI: 0.45, %HI: 75.43, HIPRed: 0.65											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
30, de novo	c.86T>C p.L29S	-3.99 del	0.00 damaging	Class C0 (GV: 234.72 - GD: 69.59)	Polymorphism (prob: 0.994)	Probably damaging (1.00/1.00)	Absent	Absent	Absent	23.5	ACMG: Likely pathogenic (PS2, PM2, PP3)

Gene: *IRX6* pLI: 0.00, %HI: 27.26, HIPred: 0.21											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
82, de novo	c.1334C>A; p.A445E	-0.27 neut	0.000 damaging	Prob damaging 1.00/0.992	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 0.966)	Absent	Absent	Absent	32	ACMG: Likely pathogenic (PS2+PM2+PP3)

Gene: *IQCA1* pLI: 0.00, %HI: 78.64, HIPred: 0.47											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
8, de novo	c.357+1G>T	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	27.10	ACMG: Likely pathogenic (PS2, PM2) Splicing predictions*

*Predicted change at acceptor site 1 bps upstream: -100.0%; MaxEnt: -100.0%; NNSPLICE: -100.0%; SSF: -100.0%;

Gene: *LIMD1* pLI: 0.13, %HI: 73.66, HIPRed: 0.414											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
57, de novo	c.87C>A; p.F29L	-3.30 del	0.00 damaging	Class C0 (GV: 251.71 - GD: 4.86)	Disease causing (prob: 0.999)	prob damaging 0.997/0.970	Absent	Absent	Absent	26.8	ACMG: Likely pathogenic (PS2+PM2+PP3)

Variants of uncertain significance

Gene: *COL4A2* pLI: 0.00, %HI: 64.22, HIPred: 0.62											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
41, de novo	c.4828C>T; p.P1610S	-7.90 del	0.00 damaging	Class C65 (GV: 0.00 - GD: 73.35)	Disease causing (prob: 1)	prob damaging 1.00/1.00	rs377451586; Absent	Allele freq: 0.00003560, 0 homozygous	EA: T=0.01% - AA: T=0.00%	25.5	ACMG: VUS (PS2+PP3)

Gene: *PHF20* pLI: 0.98, %HI 18.87, HIPred: 0.71											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
57, de novo	c.146G>A; p.R49H	-2.84 del	0.005 damaging	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	prob damaging 1.00/0.958	rs759370751, absent	Allele freq: 0.000007071 , 0 homozygous	Absent	29.10	ACMG: VUS (PS2+PP3)

Gene: *MYSM1* pLI:0.02, %HI: 43.16, HIPred: 0.64											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
82, de novo	c.1301G>A; p.R434H	-4.24 del	0.00 damaging	Class C25 (GV: 0.00 - GD: 28.82)	Disease causing (prob: 1)	Prob damaging 1.00	rs757119066; Absent	Allele freq: 0.000004079,0 homozygous	Absent	34	ACMG: VUS (PS2+PP3)

Gene: *PYGL* pLI: 0.00, %HI: 6.92, HIPRed: 0.49											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
61, de novo	c.1727G>A; p.R576Q	-3.68 del	0.00 damaging	Class C35 (GV: 0.00 - GD: 42.81)	Disease causing (prob: 1)	Probably damaging 1.000	rs267604000; Absent	Allele freq: 0.00001061, 0 homozygous	Absent	33.00	ACMG: VUS (PS2+PP3)
3, AC	c.2029A>C; p.T677P	-5.60 del	0.00 damaging	Class C35 (GV: 0.00 - GD: 37.56)	Disease causing (prob: 1)	Probably damaging 1.00 (HD & HV)	Absent	Absent	Absent	28.7	ACMG: VUS (PS2+PP3)

AC: affected child, ACMG: American College of Medical Genetics; SC: Sanger confirmed; VUS: Variant of uncertain significance

6.2.3 Familial Triome *de novo* analysis – pathogenic/likely pathogenic variants

From familial *de novo* analysis of 35 triomes, a total of 16 candidate genes were identified. Using American College of Medical Genetics (ACMG) criteria (**Figure 5-2**, **Table 5-4**), 12 *de novo* variants were classified as either "pathogenic" or "likely pathogenic" and 4 were classified as "uncertain significance".

Interestingly, 10/12 of the genes with variants classified as either pathogenic or likely pathogenic had neurological function. 8 had neurological function that can be linked to long term potentiation (LTP) or seizure mechanisms. This is further discussed in **Section 6.5.**

To consider the significance of these candidate genes to LKS, I ranked all genes with pathogenic and likely pathogenic variants using 6 further criteria: (i) whether or not the gene's function had established links to LTP or seizure mechanisms; (ii) whether or not the gene had a neurological function; (iii) whether or not the identified variant was classified as "pathogenic" via ACMG criteria; (iv) type of variant – likely loss of function variants (frame-shifts or splice-site disrupting variants) were prioritized over missense variants; (v) whether or not the gene variant was identified in a proband with classical LKS; and (vi) how strongly the gene was predicted to be haploinsufficient (**Table 6-5**).

Table 6-5: Ranking of 12 candidate genes classified as pathogenic/likely pathogenic from *de novo* analysis

Gene	Links to LTP/Sz mechanisms	Neuro-logical function	ACMG "pathogenic" variant	Likely LOF variant	Proband has classical LKS	pLI: > 0.50, %HI: <60 **AND** HIPred: >0.4	No. of criteria met
TRPC1	✓	✓	X	✓	✓	✓	5
WDFY3	✓	✓	✓	✓	X	✓	5
ERRFI1	✓	✓	X	✓	✓	X	4
RORB	✓	✓	X	X	X	✓	3
CTXN3	✓	✓	X	X	✓	X	3
KMT2A	✓	✓	X	X	X	✓	3
SOCS7	✓	✓	X	X	X	✓	3
RBM15	X	✓	X	✓	X	✓	3
NLRP3	✓	✓	X	X	X	X	2
IRX6	X	✓	X	X	✓	X	2
IQCA1	X	X	X	✓	✓	X	2
LIMD1	X	X	X	X	X	X	0

None of the 12 genes with identified pathogenic/likely pathogenic variants met all 6 criteria. *TRPC1* and *WDFY3* each met 5 criteria, and were ranked as the top candidate genes from this analysis.

6.2.3.1 TRPC1 as a candidate gene for LKS

TRPC1 encodes transient receptor potential cation channel, sub-family C, member-1. *TRPC1* is highly expressed in the brain (Martinez-Galan et al., 2018). Within the brain, TRPC1 assembles into heteromers with other TRP family members, TRPC4 and TRPC5 and functions as a G-protein coupled receptor or tyrosine kinase receptor activated calcium ion (Ca^{2+}) permeable non-selective cation channel (Rubaiy, 2019). TRPC1/4/5 have been implicated in several neuronal functions including neuronal excitability, excito-toxicity and neurite outgrowth (Schwarz et al., 2019).

There is increasing evidence that TRPC1 has an important role to play in epileptogenesis. At the cellular level, epileptiform burst firing is a basic element of epileptogenesis. It is possible that TRPC1/TRPC4 heteromeric channels may mediate epileptiform bursts and plateau potentials brought about by activation of group 1 metabotropic glutamate receptors (mGluRs). In *TRPC1/TRPC4* double knock-out mice, epileptiform bursts induced by mGluR agonists are nearly abolished (Phelan et al., 2013). *TRPC1* gene expression has also been found to be up-regulated in focal cortical dysplasia (FCD)

surgical specimens compared to control. The expression of *TRPC1* in these samples co-localised with glutamatergic and gamma-amino-butyric acid (GABA) markers, and it was postulated that *TRPC1* has a role to play in FCD epileptogenesis.

Heteromeric channels formed by TRPC1/TRPC4/TRPC5 also have roles in hippocampal synaptic neurotransmission, long term potentiation and long term depression, important mechanisms for learning and memory formation (Broker-Lai et al., 2017, Schwarz et al., 2019, Yerna et al., 2020). It has been demonstrated that in *TRPC1/TRPC4/TRPC5* triple- knock out mice, excitatory post-synaptic currents triggered by action-potentials are significantly reduced. In hippocampal slice recordings, tetanic stimulation also resulted in significantly decreased evoked post-synaptic responses. Phenotypically, *TRPC1/TRPC4/TRPC5* triple- knock out mice demonstrated deficits in spatial working memory and learning (Broker-Lai et al., 2017).

Within this cohort, I identified a novel, de novo splice-site variant (c.961-2A>C) in Case 46, a child presenting with classical LKS. He had speech and language regression at 4 years of age, associated with focal motor seizures and electrical status epilepticus in slow wave sleep (see **Appendix** for clinical summary). This variant is classified as "likely pathogenic" by ACMG criteria. It is located at the acceptor splice site of intron 6 of *TRPC1*, and is very likely to lead to skipping of exon 7.

Although, to my knowledge, *TRPC1* mutations have not previously been reported in epilepsy or neurological disorders, the close links between TRPC channel function with epileptogenesis and memory and learning have already led to investigations looking at these channels as a pharmacological target (Rubaiy, 2019).

The above evidence suggests that *TRPC1* would be a highly plausible candidate gene for an epileptic encephalopathy like LKS. Indeed, there are parallels between the functions of *GRIN2A* and the functions of *TRPC1*. Functional investigations and interrogation of other LKS cohorts will help clarify the association between *TRPC1* and LKS.

6.2.3.2 WDFY3 as a candidate gene for LKS

The gene *WDFY3* encodes WD40-repeat, FYVE Domain containing protein 3. The WD40 domain comprises 4 to 16 repeating units of 40 amino acids, usually terminating in a tryptophan-aspartic acid (W-D) di-peptide. Tandem copies of the 4 to 16 repeat units fold to form a circular solenoid protein domain (Neer et al., 1994). A common function of all WD-40 repeat proteins involves serving as a scaffold for protein interactions and coordinating multi-protein complex assemblies. WD-40 repeat proteins have been implicated in a range of generic cellular functions including transcription, signal transduction, cell-cycle regulation, apoptosis and autophagy (Stirnimann et al., 2010).

The FYVE domain is named after the 4 proteins in which it was first discovered – Fab 1, YOTB, Vac 1 and EEA1. Most FYVE domains target proteins to endosomes through binding to phosphatidylinositol-3-monophosphate (Stenmark et al., 2002).

WDFY3, also known as Autophagy linked FYVE protein, is thought to have a role in autophagy by acting as a molecular scaffold between ubiquitinated cargo and members of the autophagic system. Its FYVE domain co-localizes to phosphatidylinositol-3-monophosphate at autophagosome membranes (Dragich et al., 2016a). *WDFY3* is highly expressed in the developing central nervous system and is thought to play an important role in establishing neuronal connections and neuronal migration in the developing brain (Dragich et al., 2016a, Napoli et al., 2018a, Orosco et al., 2014).

Mutations in *WDFY3* have been implicated in a neurodevelopmental disorder characterised by autism, intellectual disability, anxiety disorder, and attention deficit hyperactivity disorder (ADHD). Most but not all individuals have abnormal head size-macrocephaly (for the majority) or microcephaly (Wang et al., 2016, Le Duc et al., 2019).

WDFY3 mutations have not previously been described in patients with seizures, LKS or epilepsy–aphasia spectrum disorders (EASD). There is, however, some phenotypic overlap between LKS and *WDFY3*- associated neurodevelopmental disorder: both are associated with autistic traits, behavioural disorders like ADHD and some intellectual disability.

Within our cohort, we have identified a pathogenic *de novo* frameshift *WDFY3* mutation in Case 30, a proband with speech and language difficulties associated with generalized tonic-clonic seizures and electrical status epilepticus in slow-wave sleep (ESES). He did not meet criteria for classical LKS as he did not have a clear history of speech and language regression. His head circumference was between the 9th and 25th centile, and his co-morbidities included autism, intellectual disability and ADHD (see **Appendix**). Overall, his phenotype can match the phenotype described for *WDFY3* neurodevelopmental disorder apart from the fact that he had epilepsy and ESES.

It is possible that epilepsy/ESES/LKS may extend the phenotypic spectrum of *WDFY3.* Indeed, the function of *WDFY3* and its already established association with a neurodevelopmental disorder suggest that it can be an attractive candidate gene for epileptic encephalopathy and LKS. However, it is important to note that this proband, Case 30, also had other genetic variants that may modify his phenotype and contribute to seizures (**Appendix**).

Further work, including interrogation of other LKS cohorts and functional work, is required to explore the role *WDFY3* may have to play in LKS.

6.2.3.3 Other genes with de novo likely pathogenic variants as candidate genes for LKS

Although the other identified genes with likely pathogenic *de novo* variants did not rank as highly as *TRPC1* or *WDFY3*, most of them have neurological functions that still make them attractive candidate genes for LKS. These genes are briefly discussed here. The clinical significance of these gene variants to LKS and to the families they were identified in, is further considered in the **Appendix**, alongside each proband's clinical summary and other genetic findings.

ERRFI1

ERRFI1 encodes a cytoplasmic protein that is up-regulated during cell-growth. Within the central nervous system, it has been established that this protein is induced by the mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) pathway during long term potentiation (LTP), and that it may have a role in the

regulation of neurite outgrowth and cortical neuron migration (Blüthgen et al., 2017, Pante et al., 2005). Within this cohort, a *de novo* frame-shift *ERRFI1* mutation, p.S189* that is absent in all control population databases, was identified in Case 53, a girl with classical LKS (clinical summary in the **Appendix**). Mutations in *ERFFI1* have not previously been reported in neurological disorders. This gene is predicted to be intolerant to haploinsufficiency by HIPred, but not by its pLI or %HI score.

Although, to my knowledge, this is the first time a variant in this gene has been discovered in LKS, this gene's function and the fact that this is a rare, likely loss-of-function variant, support the likelihood that this gene can be a plausible candidate gene for LKS. Interrogation of further LKS cohorts and functional investigations will be needed to clarify this association.

RORB

RORB encodes retinoid-orphan receptor B. It functions as a ligand- dependent transcription factor, and regulates transcriptional control of neuronal differentiation, neurogenesis and thalamic-cortical axon guidance (Liu et al., 2017a, Byun et al., 2019). Monoallelic *RORB* mutations have been associated with susceptibility to idiopathic generalised epilepsy (Rudolf et al., 2016), and developmental epileptic encephalopathy with an overlap between photosensitive generalised seizures and focal occipital seizures (Sadleir et al., 2020). Reported EEG findings include generalised spike-wave discharges, polyspike-wave discharges, photoparoxysmal responses and centro-parietal spikes. Many affected individuals have speech and language impairment (SLI), intellectual difficulties and developmental delay (Sadleir et al., 2020). As such, this phenotype has some overlap with LKS/EASD.

This study identified a novel *de novo RORB* missense variant p.C299Y in Case 57, a boy with speech and language impairment and ESES. He did not meet criteria for classical LKS as he did not have a clear history of speech and language regression (Appendix). This variant is absent in all control population databases and is predicted to be pathogenic by most in-silico prediction algorithms. It involves a highly conserved amino acid, and resides within RORB's ligand binding domain.

The above evidence suggests that it is highly likely that this variant is clinically significant for this proband. However, as this proband does not have classical LKS, and as variants in this gene have not previously been reported in LKS/EASD, the likelihood that this gene may be a candidate gene for LKS is less certain.

CTXN3

CTXN3 encodes the protein cortexin-3. The function of this gene has not been well-established. However, this gene is highly expressed in the brain and some evidence suggests that *CTXN3* may be involved in the development of GABA-ergic neurotransmission during CNS development (Šerý et al., 2015). In addition, *CTXN3* has a role in the metabolism of amyloid precursor protein (APP). APP has, in turn, been implicated in several processes involved in LTP, including synaptogenesis and regulation of the expression of the NMDA receptor N1 subunit (Šerý et al., 2015).

Haploinsufficiency prediction scores for this gene are borderline, and do not strongly support intolerance to haploinsufficiency. To date, mutations in *CTXN3* have not been identified in neurological disorders. Nonetheless, polymorphisms in this gene have been associated with schizophrenia (Šerý et al., 2015). Differential expression of *CTXN3* has also been discovered in refractory epilepsy (Liu et al., 2016).

This study identified a *de novo* missense p.P55L variant in Case 48, a boy with classical LKS (Appendix). P55 is a highly conserved amino acid in the protein's cytoplasmic domain, but the function of this domain is not known. This variant is absent in all control population databases and is highly predicted to be deleterious by all the in-silico predication algorithms queried by this study.

Overall, this gene's function and the fact that this is a rare, likely pathogenic variant, suggest that this gene can be an attractive candidate gene for LKS. Interrogation of other LKS cohorts and functional investigations will be required to further explore this possibility.

KMT2A

KMT2A encodes a histone methyltransferase enzyme which has a key epigenetic role for gene transcription during development. Within the CNS, it has been demonstrated by

several different studies that the genes regulated by *KMT2A* are involved in key pathways regulating neurogenesis, synaptic development and neuroplasticity (Kerimoglu et al., 2017, Vallianatos and Iwase, 2015).

Mutations in *KMT2A* have been described in Wiedemann Steiner Syndrome (WDSS), a syndrome characterized by global developmental delay, intellectual difficulties, speech and language impairment, short stature, dysmorphic facies and hypertrichosis. Some individuals also have autistic traits, and seizures (Chan et al., 2019). *KMT2A de novo* variants have also been identified separately in autistic spectrum disorders (Li and Pozzo-Miller, 2019) and in developmental epileptic encephalopathy (DEE), including in one individual with focal seizures and ESES (Helbig et al., 2016).

In this cohort, we have identified a *de novo* missense variant, p.R1154W in *KMT2A* in Case 58, a boy who was referred for evaluation of LKS due to fluctuating speech and language skills, and ESES. However, it emerged that, with refractory seizures, he started to have global regression of skills including cognitive and motor skills; hence, he was not regarded as having classical LKS. Review of his documented examination findings revealed features consistent with WDSS including short stature, hypertrichosis and dysmorphic features like a broad nasal bridge and tapered fingers. As such, this proband may have a phenotype that is more consistent with WDSS than LKS, and the discovery of this *de novo* p.R1154W *KMT2A* variant is likely to be highly significant for this proband. Indeed, whilst this variant is absent from all control population databases, it has already been previously identified in one other individual with WDSS and seizures. Functional investigations have shown that this variant alters *KMT2A* gene expression (Lebrun et al., 2018) Whether *KMT2A* may be a significant candidate gene for LKS, however, is less certain. This gene's function and the overlap between its associated phenotypes and LKS, would suggest that it can be a plausible candidate gene for LKS. However, *KMT2A* variants have not previously been reported in classical LKS/EASD phenotypes.

SOCS7

SOCS7 encodes suppressor of cytokine signalling protein -7. This protein inhibits cytokine signalling by linking signalling molecules to E3- ubiquitin ligases (Lawrenson et al., 2017). There is evidence that SOCS7 regulates the reelin signalling pathway through

degradation of one of its key effector proteins DAB1 (disabled-1). The reelin signalling pathway has well-established importance in maintenance in neuronal cytoarchitecture and in postnatal dendritic growth and neuroplasticity (Lawrenson et al., 2017). Whilst variants in *SOCS7* have not been reported in relation to any neurological disorders, biallelic variants in *RELN,* the gene encoding reelin have been reported to result in lissencephaly, and mono-allelic *RELN* variants have been associated with temporal lobe epilepsy with normal neuroimaging (Hong et al., 2000, Dazzo et al., 2015). This study identified a *de novo* missense variant, p.D537H, in Case 43, a child who was referred for seizures, speech and language difficulties and focal ESES. He was not classified as classical LKS, as he did not have a clear history of speech and language regression (**Appendix**). This variant is absent in all control population databases. It involves a highly conserved amino acid and lies within the protein's SOCS box domain, a domain vital for E3 ubiquitin-ligase complex recruitment (Lawrenson et al., 2017).

Considering the above evidence, it is possible that this gene variant may have, at least a contributory role to play in this proband's phenotype. The gene's function makes it a plausible candidate gene for LKS. However, variants in this gene have not previously been described in classical LKS.

RBM15

RBM15 encodes ribonucleic acid (RNA) -binding motif protein 15. This protein is a key regulator of RNA methylation and has several important roles in many cellular processes such as haematopoietic cell homeostasis (Hiriart et al., 2005). In addition, *RBM15* may have a role in neurulation and neural morphogenesis processes during early brain development (Xie et al., 2019). Variants in this gene have been implicated in various malignancies but not in neurological disorders. In this study a *de novo* frameshift *RBM15* variant, p.A393fs*11 was identified in Case 81. This boy was referred to the developmental epilepsy clinic at Great Ormond Street Hospital (GOSH DEC) for speech and language regression and behavioural concerns. However, on evaluation, his phenotype was felt to fit better with autistic regression than with classical LKS (**Appendix**). Considering that this gene has a role to play in neurodevelopment, and the fact that this is a rare, *de novo* likely loss-of-function variant, it is possible that this variant bears some clinical significance for this proband. However, as this proband does

not have classical LKS, and variants in this gene have not previously been reported in LKS/EASD/epilepsy phenotypes, there is less evidence to support *RBM15* as a candidate gene for LKS.

NLRP3

NLRP3 encodes NOD-like receptor protein-3. This protein forms part of an inflammasome complex that stimulates the release of inflammatory factors like interleukin 1β (IL-1β). Recent studies have shown that the NLRP3 inflammasome has a role in epileptogenesis and epileptic neuronal apoptosis (Shen et al., 2018). In a murine model of pilocarpine induced status-epilepticus, *NLRP3* knock-out mice had a significantly longer duration before onset of seizures than wild-type animals (Wu et al., 2019). In children undergoing surgery for refractory temporal lobe epilepsy, peripheral blood IL-1β levels were found to be significantly higher than in controls. There was also a linear correlation between IL-1β blood levels and the duration of single seizures. *NLRP3* expression was also found in temporal lobe surgical samples for these patients was also significantly higher than in control samples (Wu et al., 2019). In addition to epilepsy, dysfunction of *NLRP3* has been implicated in several other neurological disorders including Alzheimer's disease, Parkinson's disease, and Multiple Sclerosis (Eren and Özören, 2019). However, while monoallelic mutations in *NLRP3* have been reported in multi-systemic auto-inflammatory conditions (Eren and Özören, 2019), to my knowledge, *NLRP3* mutations have not been described in predominantly neurological disorders.

In this study, I identified a *de novo* missense *NLRP3* variant, p.L29S in Case 30, a child with speech and language difficulties and ESES but not classical LKS. This child also had a *de novo* variant in *WDFY3* described above (Appendix).

This variant is absent in all population databases and is predicted to be pathogenic by most in silico prediction algorithms. It involves a highly conserved amino-acid in the N-terminal pyrin domain which provides homotypic protein-protein interaction.

Considering the above evidence, it is possible that this rare variant may contribute to Case 30's phenotype. While this gene's function also makes it a plausible candidate gene for LKS, mutations in this gene have not previously been reported in classical LKS.

IRX6

IRX6 is a homeobox gene encoding a transcription factor. The function of this gene has not yet been well-studied. However, in murine models, it has been found to possibly have a role to play in neurogenesis (Cohen et al., 2000). Variants in this gene have not previously been associated with neurological disorders. This gene's %HI score predicts possible intolerance to haploinsufficiency, but its pLI score and HIPred score do not support this.

Through this analysis, I identified a *de novo* missense p.A445E variant in *IRX6* in Case 82, a child with classical LKS (**Appendix**). This variant is absent from all control population databases, it involves a highly conserved amino acid, and is predicted to be deleterious by 3/5 in silico prediction algorithms queried by this study.

This proband has only 2 genetic variants identified in this study (**Appendix).** Considering the possible neurological function of this gene, this genetic variant is more likely to have a contributory role to his phenotype/LKS than his other identified variant of uncertain significance. Better understanding of the function of this gene and further work including interrogation of other LKS cohorts and functional work will be required to establish if this gene has any causative/contributary role to play in LKS.

IQCA1

IQCA1 encodes an ATPase (adenosine triphosphate-ase) that is a component of the nexin-dynein regulatory complex. It may have a role in the regulation of ciliary motility and the maintenance of the distal axoneme (Bower et al., 2013). Although it is relatively highly expressed in the brain, its role in the CNS has not been established. Variants in this gene have not been reported in any disorders. The gene's haploinsufficiency prediction scores suggest that it may be tolerant to haploinsufficiency.

In this study, a *de novo* splice-site variant was identified in Case 8, a girl with classical LKS (Appendix). This variant is absent from all control population databases and is highly predicted to disrupt splicing and lead to exon 2 skipping.

Considering that this gene is highly expressed in the brain, and that this is a rare, likely loss-of-function variant, it is possible that this gene variant may have at least a

contributory role to play in this child's phenotype/LKS. Clarification on the function of this gene and further work including interrogation of other LKS cohorts and functional investigations will be required to ascertain this.

LIMD1

LIMD1 encodes LIM-domain containing protein-1. This protein is a transcription regulator that is involved in cell adhesion, cytoskeletal organisation, cell morphology and cell migration (Bai et al., 2011). It has a low level of expression in the brain but its function in the CNS is not well-characterised. This gene's haploinsufficiency scores are borderline, suggesting that it may tolerate haploinsufficiency. However, there has been one report of a monoallelic copy number variant in *LIMD1* in an individual with focal epilepsy and developmental delay (Howell et al., 2013). In addition, a monoallelic missense *LIMD1* variant, p.S255R, was identified as one of many rare, potentially deleterious variants in a family with temporal lobe epilepsy and aphasic seizures. It should be noted that this latter family also carried a likely deleterious *RELN* mutation, a gene that has better established association with temporal lobe epilepsy (Dazzo et al., 2015).

In this study, a *de novo* p.F29L *LIMD1* missense variant was identified in Case 57, a proband with speech and language impairment and ESES. He did not meet criteria for classical LKS as he did not have a clear history of speech and language regression. This individual also had a likely pathogenic *de novo* variant identified in *RORB* described above and a *de novo* variant of uncertain significance in *PHF20* (**Appendix**).

The p.F29L variant is absent from all population databases. It involves a moderately conserved amino acid and is predicted by most-in silico prediction algorithms to be deleterious.

Although the function of this gene in the CNS is not well understood, considering that this is a *de novo* likely pathogenic variant, and the fact that there have been isolated reports of variants in this gene linked to similar phenotypes, there is some possibility that this variant may have some contribution to this patient's symptoms. However, whilst there is also some overlap between phenotypes previously associated with this

gene and LKS, there is scarce other evidence to support this gene as a candidate gene for classical LKS.

6.2.4 Familial Triome *de novo* analysis – variants of uncertain significance

In this analysis, *de novo* variants of unknown significance were identified in 4 other genes: *COL4A2, PHF20, MYSM1 and PYGL.* All of these variants had rare occurrence in control population databases.

COL4A2 which encodes the α2 chain of type IV collagen is associated with focal seizures in the context of cerebral malformations e.g. porencephaly. *PHF20* identified in Case 57 (who also has *de novo* variants in *RORB* and *LIMD1* described above) may have a role in transcriptional regulation of genes involved in neurogenesis and synaptic development. The function of the 2 remaining genes, *MYSM1* and *PYGL,* within the CNS is unclear.

In addition to a *de novo PYGL* variant in Case 61, a girl with classical LKS, an autosomal dominant inherited variant in *PYGL* was also identified in a father and son who both have LKS.

The significance of these gene variants to these families and to LKS, as a phenotype, are considered in the **Appendix**, alongside the clinical features and all other genetic findings, for each proband.

6.3 Familial Triome Analysis Using a Recessive/X-linked recessive Model

6.3.1 Methods

For each of the 38 triomes, genetic analysis using a recessive disease model was also undertaken in order to identify homozygous and compound heterozygous variants. Additionally, for all male probands, genetic analysis using an X-linked recessive model was carried out.

Each triome was analysed individually, one at a time. For each triome, WES/WGS data was uploaded in the form of variant call files to Qiagen's Ingenuity Variant Analysis (QIVA) platform. A similar set of pre-determined filters with slightly different parameters was used to remove low confidence variants, common variants, and likely benign variants (**Table 6-6**).

For all 38 triomes, a customized genetic analysis filter was added to screen for homozygous variants **(Table 6-7**). The process in the paragraph above was then repeated and a different two-tier customised genetic analysis filter was applied for compound heterozygous variants (**Table 6-8**). Lastly, for X-linked recessive analysis for all male probands, the process in the paragraph above was repeated once more; this time, QIVA's physical location filter was applied to keep only variants located within the X-chromosome. A customised genetic analysis filter was then applied for X-linked recessive inherited variants (**Table 6-9**).

Table 6-6: QIVA pre-determined filters for recessive variants

CONFIDENCE FILTERS: Include only:-
(i) Call quality ≥ 20 in any case and any control (ii) Read depth ≥ 10 in any case and any control (iii) Allele fraction ≥ 15 in any case and ≥ 15 in any control (iv) Outside top 5% of most exonically variable 100 bases window
COMMON VARIANTS FILTERS: unless an established pathogenic variant, exclude:-
(i) Variants that are present in 1000G with an allele frequency of > 0.1% (ii) Variants that are present in ExAC with an allele frequency of > 0.1% (iii) Variants that are present in gnoMAD with an allele frequency of > 0.1% (iv) Variants that are present in ESP with an allele frequency of > 0.1%
PREDICTED DELETERIOUS FILTERS: keep only variants that are:
(i) no more than 20 bases into the intron (ii) Pathogenic/Likely pathogenic according to ACMG guidelines (iii) Listed in Human Gene Mutation Database
OR:-*are predicted to be associated with loss of gene function or alteration of gene function:-* (i) frameshift, in-frame indel, or start/stop codon change (ii) missense CADD score > 20 (iii) splice site loss up to 10 bases into intron or as predicted by MaxEnt Scan*

Table 6-7: Genetic Analysis Filter for Homozygous Variants

Use recommended settings for: **recessive variants** Restrict to **transmitted variants**.	
CASE (affected patient) samples. **Keep only** variants which are:-	CONTROL (parental) Samples. **Keep only** variants which are:-
Homozygous	Heterozygous
AND:-	AND:-
The genotypes selected above occur in at least **1** of the 1 case samples (100%) at variant level	The genotypes selected above occur in at least **2** of the 2 control samples (50%) at variant level

Table 6-8: Two-tier Genetic Analysis Filters for Compound Heterozygous Variants

Genetic Analysis Filter Tier 1 Use recommended settings for: **recessive variants** Restrict to **transmitted variants**.	
CASE (affected patient) samples. **Keep only** variants which are:-	CONTROL (parental) Samples. **Exclude** variants which are:-
Compound heterozygous	Homozygous
Heterozygous	Heterozygous
AND:-	AND:-
The genotypes selected above occur in at least **1** of the 1 case samples (100%) at **variant** level	The genotypes selected above occur in at least **2** of the 2 control samples (50%) at **variant** level
Genetic Analysis Filter Tier 2 Use recommended settings for: **recessive variants.** Restrict to **transmitted variants**.	
CASE samples. **Keep only** variants which are:-	CONTROL (parental) Samples. **Keep only** variants which are:-
Homozygous	Heterozygous
Compound heterozygous	
Haploinsufficient	
Hemizygous	
Heterozygous	
Het ambiguous	
AND:-	AND:-
The genotypes selected above occur in at least **1** of the 1 case samples (100%) at **variant** level	The genotypes selected above occur in at least **2** of the 2 control samples (50%) at **gene** level

Table 6-9 Genetic Analysis for X-linked Recessive Variants

Use recommended settings for: **recessive variants**.	
CASE (affected patient) samples. **Keep only** variants which are:-	CONTROL (parental) Samples. **Keep only** variants which are:-
Homozygous	Heterozygous
AND:-	AND:-
The genotypes selected above occur in at least **1** of the 1 case samples (100%) at variant level	The genotypes selected above occur in at least **1** of the 2 control samples (50%) at variant level

Following filtering by the Ingenuity Variant Analysis Platform, the resultant variant list for each analysis was exported into a Microsoft Excel spreadsheet for further manual analysis.

During manual analysis, criteria similar to the ones used for *de novo* analysis were used to filter out further variants (**Table 6-10**). As haploinsufficiency predictors would be less relevant for this analysis, this criterion was left out.

Table 6-10 Manual filters for recessive variants

Discarded variants	**Method**
Homozygous occurrence in any control population database	Checking population databases including gnoMAD, ExAC, 1000 genomes project, ESP, and dbSNP
CADD score of < 20 unless the variant is within a splice site region or is a frame-shift variant	The utility of the CADD score was introduced in Chapter 5 (**Table 5-3**).
Predicted to be benign by in-silico prediction algorithms.	Each variant was put through five *in silico* prediction algorithms to determine the likelihood of pathogenicity (SIFT, PROVEAN, GVGD, Mutation Taster and PolyPHEN). This was partly facilitated by the use of AVS. The in-silico prediction algorithms used were introduced in Chapter 5 (**Table 5-3**). Only variants predicted to be pathogenic by 3 or more different *in silico* prediction algorithms were kept for downstream analysis
Variants in splice site regions that were not predicted to lead to splicing defects	Splicing defect predictions from the splice site algorithms, Neural Network Splice (NNSplice), Splice Site Finder- like (SSF), and MaxEntScan (introduced in **Chapter 5.4.1, Table 5 3)** were derived using AVS. A combination of a percentage difference of 15% or more for MaxEntScan and 5% or more for NNSplice and SSF was taken to imply a likely splice site disruption.
Genes not expressed in the brain	Gene expression within the brain was checked using data from the UK Brain Expression Consortium (http://www.braineac.org) and data from the Genotype Tissue Expression project (https://gtexportal.org/home)

AVS: Alamut Visual software (https://www.interactive-biosoftware.com).

The American College of Medical Genetics and Genomics (ACMG) guidelines were again used to aid interpretation of the remaining sequence variants (Richards et al., 2015).

For relevant genetic variants, direct Sanger sequencing was performed to verify that these were true findings and to confirm that all compound heterozygous variants occurred in *trans*.

6.3.2 Results – Recessive Analysis

No significant homozygous gene variants were identified. Apart from the *SMC1A* variant already detected in the Epilepsy gene panel analysis, there were no other significant findings from X-linked recessive analysis.

Compound heterozygous variants were identified in 4 genes. These are presented in (**Table 6-11).**

Table 6-11: Candidate genes with compound heterozygous variants identified from recessive model analysis

Gene: *PLEKHG2*											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
43, UF	c.2284C>T; p.R762C	-3.51 del	0.00 damaging	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	Possibly damaging 0.912/Benign 0.229	rs61730569; Absent	Allele freq: 0 0.0003089, 0 homozygous	EA: T=0.05% - AA: T=0.02%	33	ACMG: VUS (PM2+PP3)
43, UM	c.2465G>A; p.R822Q	-1.42 neutral	0.00 damaging	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 0.604)	Prob damaging 0.999/possibly damaging 0.852	rs147184413; MAF<0.01	Allele freq: 0.0001387, 0 homozygous	EA: A=0.00% - AA: A=0.07%	34	ACMG: VUS (PM2+PP3)

Gene: *ADAMTSL4*											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
52, UF	c.2848G>C; p.G950R	-7.36 del	0.004 damaging	Class C65 (GV: 0.00 - GD: 125.13)	Disease causing (prob: 1)	Probably damaging 1.00/1.00	Absent	Absent	Absent	27.6	ACMG: VUS (PM2+PP3)
52, UM	c.190G>A; p.V64M	-1.18 neut	0.002 damaging	Class C0 (GV: 234.99 - GD: 0.00)	Disease causing (prob: 0.947)	Probably damaging 0.996./0.923	rs754048794; Absent	Allele freq: 0.00002413, 0 homozygous	Absent	23.9	ACMG: VUS (PM2+PP3)

Gene: *VPS13A*											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
50, UF	c.5587G>A; p.A1863T	-2.69 del	0.004 damaging	prob damaging 0.999/0.949	Class C0 (GV: 232.67 - GD: 0.00)	Disease causing (prob: 1)	rs146389747, absent	Allele freq: 0.00006746; 0 homozygous	Absent	33	ACMG: VUS (PM2+PP3)
50, UM	c.2888C>T; p.T963M	-2.48 neutral	0.010 damaging	Class C0 (GV: 353.86 - GD: 0.00)	Disease causing (prob: 1)	prob damaging 1.00/0.920	rs577694390; MAF< 0.01	Allele freq: 0.00002831, 0 homozygous	Absent	33	ACMG: VUS (PM2+PP3)

Gene: *ABCA7*											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/Others
61, UF	c.2639G>A; p.R880Q	-3.77 del	0.001 damaging	Class C35 (GV: 0.00 - GD: 42.81)	Disease causing (prob: 0.997)	Prob damaging 0.999/0.977	rs143718918 MAF< 0.01	Allele freq: 0.001116, 0 homozygous	EA: A=0.09% - AA: A=0.00%	28.6	ACMG: VUS (PM2+PP3)
61, UM	c.2966G>A; p.R989H	-4.55 del	0.001 damaging	Class C0 (GV: 241.31 - GD: 1.62)	Disease causing (prob: 1)	prob damaging 1.00/0.996	rs139214131; Absent	Allele freq: 0.0007560, 0 homozygous	EA: A=0.09% - AA: A=0.02%	27.9	ACMG: VUS (PM2+PP3)

ACMG: American College of Medical Genetics; APS: Awaiting parental sequencing, UF: inherited from unaffected father, UM: inherited from unaffected mother

6.3.3 Familial Triome autosomal recessive analysis – variants of uncertain significance

For this analysis, following filtration, 4 genes were identified with compound heterozygous variants occurring in *trans*. All the identified gene variants were classified as variants of uncertain significance by American College of Medical Genetics (ACMG) guidelines.

The significance of these gene variants to these families and to LKS, as a phenotype, are considered in the Appendix, alongside the clinical features and all other genetic findings, for each proband.

ADAMTSL4 is the only gene identified in this category without a clear function in the CNS. The other 3 genes have interesting neurological functions briefly mentioned here.

ABCA7

ABCA7 encodes adenosine triphosphate (ATP)- binding cassette sub-family A member 7. It is involved in lipid transport and is responsible for maintaining intracellular lipid metabolism and cellular homeostasis (Surguchev and Surguchov, 2020). This gene has been well-studied for its possible role in the pathogenesis of Alzheimer's disease. This protein may have a role to play in long-term potentiation (LTP), as it is also involved in the processing of amyloid precursor protein (APP). In addition, it has a role in the phagocytosis of amyloid beta aggregates. APP is involved in many physiological processes involving LTP, including neurite outgrowth, and synapse formation. Amyloid beta is also known to have a significant role in mediating hippocampal LTP (Aikawa et al., 2018). Low physiological concentrations of amyloid beta are necessary for LTP and memory, while high concentrations of amyloid beta peptide inhibit hippocampal synaptic plasticity (Ricciarelli and Fedele, 2018).

In this cohort, compound heterozygous variants in *ABCA7* were identified in Case 61, a girl with classical LKS and above average intelligence (**Appendix**). Both variants are rare and involve highly conserved amino-acids in the first nucleotide binding domain of ABCA7 which is important for its transport activity.

Although both these gene variants have been classified as variants of uncertain significance, this gene's function makes it a plausible candidate gene to consider for this family and LKS.

VPS13A

VPS13A encodes vacuolar protein sorting 13 homolog A, also known as chorein. This protein. Chorein binds to phosphatidyinositol lipids of the cell membrane and contributes to directing vesicle trafficking. It has been found to have roles in neurotransmitter release, neuronal cell survival, autophagy and mitochondrial function (Lang et al., 2017).

Biallelic mutations in *VPS13A* have been reported in choreo-acanthocytosis, a rare adult-onset disease, characterized by a progressive movement disorder and erythrocyte acanthocytosis. Co-morbidities include epileptic seizures, cognitive impairment and behavioural difficulties (Lang et al., 2017). Recently, a homozygous variant in *VPS13A* was also reported in a consanguineous family with epilepsy without a clinically apparent movement disorder. However, for affected individuals, FDG-PET and FP-CIT-SPECT scans showed abnormalities in striatal glucose metabolism and loss of striatal dopamine transporter availability respectively (Weber et al., 2018).

In this cohort, compound heterozygous *VPS13A* variants were identified in Case 50. Both variants are extremely rare and are predicted to be deleterious by most in-silico prediction algorithms. Case 50 is a girl with congenital nystagmus, who was referred to GOSH DEC for refractory seizures with ESES. As she had global regression of developmental skills rather than a predominantly language-led regression, she was not classified as having typical LKS. Review of documented examination findings during active disease revealed significant motor difficulties including dyspraxia, ataxia and tremor. In her teenage years, due to increasing difficulty with movement coordination, she was wheelchair dependent. As her seizures came under better control, however, she recovered her motor skills. Presently at 20 years of age, she is not reported to have significant motor difficulties **(Appendix)**.

The function of this gene suggests it can be considered as a candidate gene for both Case 50's presentation and LKS. However, previously described *VPS13A* phenotypes

may have more of an overlap with Case 50's presentation than with classical LKS. Therefore, whilst it is possible that these gene variants may have a contributory role to play in Case 50's phenotype, there is less evidence to support this gene as a candidate gene for LKS.

PLEKHG2

PLEKHG2 encodes a Rho guanidine exchange factor (GEF). In the CNS, its functions include actin cytoskeleton arrangement, neuronal network formation, dendritic dendritic spine formation and arborization (Edvardson et al., 2016).

A homozygous mutation in this gene has been reported in 2 unrelated families with a phenotype characterised by intellectual difficulty, dystonia, acquired microcephaly and white matter abnormalities on neuroimaging. Only 1 affected individual had a history of epileptic seizures (Edvardson et al., 2016)

In this study, compound heterozygous variants in *PLEKHG2* were identified in Case 43, a child with speech and language difficulties and focal ESES. He did not meet criteria for classical LKS as he did not have a clear history of speech and language regression. His other genetic findings include a chromosome 1p duplication identified on diagnostic microarray and a *de novo* likely pathogenic variant in *SOCS7* described above (**Appendix**). He did not have any documented examination findings of dystonia or movement disorder. His head circumference documented at 5 years 10 months of age was between the 2nd and 9th centile. A magnetic resonance imaging (MRI) brain scan performed at 6 years of age was unremarkable.

The function of this gene makes it a plausible candidate for both this proband's phenotype and for LKS. However, apart from intellectual difficulties, there are limited similarities between the phenotype described by Edvardson et al, and Case 43's phenotype or LKS. Overall, there may be a some possibility these gene variants may contribute to Case 43's phenotype. However, at present, there is limited evidence to support this gene as a candidate gene for LKS.

6.4 Autosomal Dominant Analysis – Genes with variants found in 3 or more families

Considering that a significant proportion of reported *GRIN2A* mutations within LKS/EASD were inherited from unaffected parents, to search for candidate genes with incomplete penetrance, I performed a final analysis to identify genes in which autosomal dominant- inherited variants occurred frequently (in 3 or more families) within this LKS cohort. As the significance of inherited variants in genes without known neurological function would be difficult to interpret, only genes with known neurological function, particularly in relation to seizure or long term potentiation (LTP) mechanisms, were considered.

6.4.1 Methods – Genes with variants in 3 or more families

WES/WGS data for the entire cohort- 56 patient (case) and 80 parental (control) samples- was uploaded in the form of variant call files to Qiagen's Ingenuity Variant Analysis (QIVA) platform. A set of pre-determined filters with customised parameters was then used to remove low confidence variants, common variants, and likely benign variants (**Table 6-12**).

A customized genetic analysis filter was added to elicit genes with significant variants in 3 or more probands (**Table 6-13**).

The resultant variant list after QIVA filtering was then exported into a Microsoft Excel spreadsheet for further manual analysis.

During manual analysis, criteria similar to the ones used for *de novo* analysis were used to filter out further variants (**Table 6-14**). One additional criterion was added- the function of the genes identified was checked, through literature review, and genes without a clear function in the central nervous system (CNS) were disregarded.

Table 6-12 QIVA pre-determined filters for genes with variants in 3 or more families

CONFIDENCE FILTERS: Include only:-
(i) Call quality ≥ 20 in any case and any control (ii) Read depth ≥ 10 in any case and any control (iii) Allele fraction ≥ 35 in any case and ≥ 10 in any control* (iv) Outside top 5% of most exonically variable 100 bases window
COMMON VARIANTS FILTERS: unless an established pathogenic variant, exclude:-
(i) Variants that are present in 1000G with an allele frequency of > 0.01% (ii) Variants that are present in ExAC with an allele frequency of > 0.01% (iii) Variants that are present in gnoMAD with an allele frequency of > 0.01% (iv) Variants that are present in ESP with an allele frequency of > 0.01%
PREDICTED DELETERIOUS FILTERS: keep only variants that are:
(i) no more than 20 bases into the intron (ii) Pathogenic/Likely pathogenic according to ACMG guidelines (iii) Listed in Human Gene Mutation Database
OR:-*are predicted to be associated with loss of gene function or alteration of gene function:-* (i) frameshift, in-frame indel, or start/stop codon change (ii) missense CADD score > 20 (iii) splice site loss up to 10 bases into intron or as predicted by MaxEnt Scan*

* Allele fraction is set lower for controls because the following filter for genetic analysis is set to exclude certain variants from controls. If allele fraction is set too high, QIVA will only exclude variants with allele fraction > the set value and fail to exclude those with lower allele fraction.

Table 6-13 Genetic analysis filters for genes with variants in 3 or more families

Use recommended settings for: **dominant variants** **No restrictions**	
CASE (affected patient) samples. **Keep only** variants which are:-	CONTROL (parental) Samples. **Exclude** variants which are**:-**
Homozygous/Compound heterozygous	Homozygous/ Compound heterozygous
Haploinsufficient	Haploinsufficient
Hemizygous	Hemizygous
Het-ambiguous	Het-ambiguous
Heterozygous	Heterozygous
AND:-	AND:-
The genotypes selected above occur in at least **3** of the 56 case samples (5%) at **gene** level	The genotypes selected above occur in at least **8** of the 80 control samples (10%) at **variant** level*

*Variants which occur too frequently in the control sample (arbitrarily set at 10%) were excluded on the basis that these would likely be benign polymorphisms.

Table 6-14 Manual analysis filters for genes with variants in 3 or more families

Discarded variants	Method
Homozygous occurrence in any control population database	Checking population databases including gnoMAD, ExAC, 1000 genomes project, ESP, and dbSNP
CADD score of < 20 unless the variant is within a splice site region or is a frame-shift variant	The utility of the CADD score was introduced in Chapter 5 (**Table 5-3**).
Predicted to be benign by in-silico prediction algorithms.	Each variant was put through five *in silico* prediction algorithms to determine the likelihood of pathogenicity (SIFT, PROVEAN, GVGD, Mutation Taster and PolyPHEN). This was partly facilitated by the use of AVS. The in-silico prediction algorithms used were introduced in Chapter 5 (**Table 5-3**). Only variants predicted to be pathogenic by 3 or more different *in silico* prediction algorithms were kept for downstream analysis
Variants in splice site regions that were not predicted to lead to splicing defects	Splicing defect predictions from the splice site algorithms, Neural Network Splice (NNSplice), Splice Site Finder- like (SSF), and MaxEntScan (introduced in **Chapter 5.4.1, Table 5 3)** were derived using AVS. A combination of a percentage difference of 15% or more for MaxEntScan and 5% or more for NNSplice and SSF was taken to imply a likely splice site disruption.
Variants in genes that were predicted to tolerate haploinsufficiency	3 methods were used to predict haploinsufficiency: the HIPred score, the haploinsufficiency index (%HI) and the pLI score. These were introduced in **Table 5-7**. Heterozygous variants in genes that met at least 1 out of 3 of the following criteria: (i) pLI: > 0.50; %HI < 60, HIPred: > 0.40, were kept.
Genes not expressed in the brain	Gene expression within the brain was checked using data from the UK Brain Expression Consortium (http://www.braineac.org) and data from the Genotype Tissue Expression project (https://gtexportal.org/home)
Genes without established neurological function	Gene function was checked through literature review on PubMed

6.4.2 Results- Genes with variants in 3 or more families

4 genes were identified, meeting all the above criteria. These genes are presented in **Table 6-15**.

Table 6-15 Candidate genes with variants identified in 3 or more families

Gene: *MYH7B* pLI: 0.00; %HI: 63.11; HIPRed: 0.78											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/ Others
62, APS	c.716T>C p.V239A	-3.05 del	0.000 damaging	Class C65 (GV: 0.00 - GD: 65.28)	Not available	possibly damaging 0.951/0.769	rs767776965; Absent	Allele freq: 0.0000324, 0 homozygotes	Absent	23.7	ACMG: VUS (PM6+PP3)
16, APS	c.2433+5G>A	N.A.	N.A.	N.A.	N.A.	N.A.	rs370871561	Allele freq: 0.0000040, 0 homozygotes	EA: A=0.01% - AA: A=0.00%	14.0	ACMG: VUS (PM6+PP3) Splicing predictions*
64, AUM	c.5303delA p.Q1768fs*6	N.A.	N.A.	N.A.	N.A.	N.A.	Absent	Absent	Absent	-	ACMG: VUS (PM2+ PM6+PP3) Splicing predictions†
8, UM	c.5201T>G p.L1734R	-4.11 del	0.001 damaging	Class C65 (GV: 0.00 - GD: 101.88)	-	possibly damaging 0.947/0.817	rs758629702; Absent	Allele freq: 0.0000116, 0 homozygotes	Absent	25.5	ACMG: VUS (PP3)

*Predicted change at donor site 5 bps upstream: MaxEnt: -100.0%; NNSPLICE: -96.6%; SSF: -16.3%;

†Predicted change at donor site 2 bps downstream: MaxEnt: -56.8%, NNSPLICE: -74.3%; SSF: -14.9%

Gene: *DOCK8* pLI: 0.00, %HI: 35.68, HIPRed: 0.42											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/ Others
3, AC	c.5602G>C p.V1868L	-2.79 del	0.00 damaging	Class C0 (GV: 223.30 - GD: 30.92)	Disease causing (prob: 1)	probably damaging 0.999/0.999	rs375218876; Absent	Allele freq: 0.0000460, 0 homozygotes	EA: C=0.00% - AA: C=0.02%	29.1	ACMG: VUS (PP1+PP3)
74, UM	c.5775C>G p.F1925L	-5.56 del	0.000 damaging	Class C0 (GV: 245.62 - GD: 4.86)	Disease causing (prob: 1)	probably damaging 0.995/0.971	rs376877796; Absent	Absent	EA: G=0.01% - AA: G=0.00%	29.5	ACMG: VUS (PP3)
44, UM	c.3197G>T p.G1066V	-8.22 del	0.001 damaging	Class C15 (GV: 87.31 - GD: 69.84)	Disease causing (prob: 1)	possibly damaging 0.896/0.768	rs745488546; Absent	Allele freq: 0.0000040, 0 homozygotes	Absent	25	ACMG: VUS (PP3)

Gene: *NOTCH1* pLI: 1.00 %HI: 0.15, HIPRed: 0.82											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/ Others
8, UF	c.949G>A p.G317S	-5.14 del	0.008 damaging	Class C55 (GV: 0.00 - GD: 55.27)	Disease causing (prob: 1)	probably damaging 1.00/1.00	rs749696049; Absent	Allele freq: 0.0000040, 0 homozygotes	Absent	27.9	ACMG: VUS (PP3)
53, UM	c.2182G>A p.G728R	-6.86 del	0.001 damaging	Class C65 (GV: 0.00 - GD: 125.13)	Disease causing (prob: 1)	probably damaging 0.998/0.959	rs771222092; Absent	Allele freq: 0.0000093, 0 homozygotes	Absent	23.8	ACMG: VUS (PP3)
7, UM	c.2835C>G p.D945E	-2.45 neut	0.280 tol	Class C35 (GV: 0.00 - GD: 44.60)	Disease causing (prob: 1)	probably damaging 1.00/0.997	rs756174213; Absent	Allele freq: 0.0000161, 0 homozygotes	Absent	24.1	ACMG: VUS (PP3)

Gene: *LAMA5* pLI: 0.94; %HI: 63.60; HIPRed: 0.68											
Patient number/ Inheritance	Mutation	Provean	SIFT	GVGD	Mutation taster	Polyphen	dbSNP/ 1000 Genomes	Gnomad	EVS	CADD	ACMG classification/ Others
86, APS	c.4906C>T p.R1636C	-5.86 del	0.070 tolerated	Class C0 (GV: 257.44 - GD: 11.33)	Disease causing (prob: 1)	probably damaging 0.999/possibly damaging 0.828	rs771601969; Absent	Allele freq: 0.0000355, 0 homozygotes	Absent	26.1	ACMG: VUS (PM6+PP3)
50, UM	c.4879C>T p.R1627C	-4.21 del	0.018 damaging	Class C0 (GV: 246.14 - GD: 50.17)	Disease causing (prob: 1)	probably damaging 1.00/0.925	rs139955074; Absent	Allele freq: 0.0000497, 0 homozygotes	EA: A=0.01% - AA: A=0.00%	33	ACMG: VUS (PP3)
81, UF	c.4268C>T p.A1423V	-3.05 del	0.008 damaging	Class C0 (GV: 141.92 - GD: 35.60)	Disease causing (prob: 1)	probably damaging 0.988/possibly damaging 0.815	rs750949736; Absent	Allele freq: 0.0000120; 0 homozygotes	Absent	23.5	ACMG: VUS (PP3)

ACMG: American College of Medical Genetics; APS: Awaiting parental sequencing, UF: inherited from unaffected father, UM: inherited from unaffected mother

6.4.3 Genes with variants in 3 or more families – variants of uncertain significance

Most of the variants elicited by this analysis were dominantly inherited and all of them were classified by American College of Medical Genetics guidelines as variants of uncertain significance.

MYH7B

Within this cohort, *MYH7B* is the gene in which rare variants occurred most frequently. This gene encodes myosin heavy chain 7B. This actin binding protein has been found to have important roles in regulating neuronal dendritic spine structure and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor trafficking in the hippocampus – both essential processes in long term potentiation. Studies have shown that reducing the expression of *MYH7B* in mature neurons in culture result in large, abnormally shaped heads with many filopodia-like protrusions. In addition, these neurons had impaired excitatory post-synaptic current (EPSC) amplitudes, along with a decrease in the density of AMPA receptors, attributed to alterations in the actin cytoskeleton (Rubio et al., 2011).

MYH7B is most highly expressed in the heart, brain and testis. *MYH7B* variants have not been previously described in relation to neurological disorders, however, a recent study has associated loss-of-function *MYH7B* variants in a few families (1.49%) with hypertrophic cardiomyopathy. All the missense variants identified in this study resided within the myosin tail domain of the protein, believed to provide the structural backbone of the thick filament (Chen et al., 2020b).

This study has uncovered *MYH7B* variants in 4 probands. The first is a frame-shift variant, p.Q1768fs*6, that is absent from all population databases and may possibly be *de novo* – the variant was absent from maternal sample and paternal DNA was not available. The second is a rare splice-site variant c.2433+5G>A, that may possibly be *de novo* pending parental DNA sequencing. In addition, 2 further rare missense variants were identified. Both were predicted by most in-silico prediction algorithms to be deleterious – one, p.V239A, may possibly be *de novo* pending parental sequencing and

one was inherited p.L1734R from an unaffected mother. V239 is a highly conserved amino-acid in the myosin head domain of MYH7B. This domain is believed to be important for ATPase activity and interaction with actin. L1734 lies in the myosin tail domain. None of these probands have had cardiovascular symptoms.

The function of this gene makes *MYH7B* an attractive candidate for a phenotype like LKS. It is likely that these gene variants may have, at least, a contributory role to play in the phenotypes of these probands/LKS. This is further discussed in the **Appendix** alongside each proband's clinical summary and other genetic findings.

DOCK8

DOCK8 encodes dedicator of cytokinesis 8, a guanine nucleotide exchange factor (GEF) that activates the Rho-GTPase, CDC42. This protein is best studied for its role in the immune system, where it has been found to regulate dendritic cell migration. Within the CNS, studies have shown that *DOCK8* mediates microglial migration and phagocytosis in neuro-inflammation and neurodegeneration (Namekata et al., 2019). It may also be possible that like other members of the DOCKC group, to which *DOCK8* belongs, *DOCK8* may have a role in neuronal axon growth, through its interaction with CDC42 (Griggs et al., 2008).

Biallelic variants in *DOCK8* result in combined immunodeficiency and hyper-immunoglobin E syndrome (Gadea and Blangy, 2014). Conversely, rare, monoallelic structural variants in *DOCK8* have been associated with intellectual disability (Griggs et al., 2008) and autistic spectrum disorder (Wang et al., 2016). Many affected individuals also have speech and language impairment and prominent behavioural disorders (Krgovic et al., 2018).

This study has identified rare variants in *DOCK8* in 3 families. All these probands have classical LKS. 1 variant, p.V1868L is shared between a father and son pair with LKS. The other 2 variants, p.F1925L and p.G1066V were inherited from unaffected mothers. All 3 variants involve highly conserved amino acids. F1868 and F1925 lie within the DHR-2 (Dock homology region-2) domain of the protein, important for its GEF function.

DOCK8's function and its previous association with neurodevelopmental disorders, suggest it may have a contributory role to play in LKS. The contribution these variants may have to these probands and to LKS as a phenotype is further discussed in the **Appendix.**

NOTCH1

NOTCH1 encodes a single-pass transmembrane receptor protein involved in cell-signalling. NOTCH signalling is important for many events in CNS development, including neural stem cell maintenance, cell fate determination and regulation of neurite outgrowth. In addition NOTCH signalling has been found to be involved in N-methyl-D-aspartate (NMDA) receptor downstream synaptic potentiation, and epileptogenesis, likely through dendritic spinogenesis and/or through facilitation of synaptic vesicle neurotransmitter release (Sha et al., 2014).

Monoallelic mutations in *NOTCH1* have been reported in Adams-Oliver syndrome (AOS), a developmental disorder characterised by cutis aplasia, transverse limb abnormalities and cardiac abnormalities. Some individuals have neurodevelopmental delay including intellectual difficulties, and some have seizures (Stittrich et al., 2014, Southgate et al., 2015).

In this cohort, 3 rare *NOTCH1* missense variants have been identified. All of them have been inherited from unaffected parents. All of these probands have classical LKS and none of these probands have characteristic AOS features. However, I note that AOS variants were mostly protein-truncating variants. The few missense variants reported in AOS occurred in the ligand binding domain. Only 1 reported AOS missense variant occurred within a non-calcium binding part of NOTCH1's extracellular epidermal growth factor- like (EGF-like) domain. All the missense variants identified in this LKS cohort occur within calcium-binding parts of the EGF-like domain, believed to be important for initiation of certain cell-signalling processes. It is possible that AOS variants may result in a more severe phenotype.

The function of *NOTCH-1* and the identification of rare variants in 3 families in this cohort, make it plausible that this gene may have a contributory role to play in the phenotypes of these probands and to LKS. This is further considered in the **Appendix**.

LAMA5

LAMA5 encodes laminin alpha-5, an extracellular matrix glycoprotein. This protein is deposited within the developing brain, as synaptic structure transitions from dynamic to stable, and has critical roles in postnatal dendritic spine regulation and synapse stability. Disruption in *LAMA5* in hippocampal neurons has been shown to lead to alterations in dendritic spine size and synapse-loss. In addition, *LAMA5*-knock-out mice fail to discriminate between novel and familiar objects in object recognition tasks (Omar et al., 2017).

Biallelic variants in *LAMA5* have been associated with multi-systemic syndromic disorders often affecting the skin, kidneys and limbs in addition to the CNS (Jones et al., 2020). A single *de novo* monoallelic splice-site disrupting variant has also been previously reported in an individual with neurodevelopmental delay, speech and language impairment, autistic traits and seizures (Han et al., 2018).

Inherited missense variants have been identified in 3 families within this cohort. None of these families have classical LKS. The function of *LAMA5* suggests it may have a contributory role to play in the phenotypes of these probands. This is further discussed in the **Appendix** with their clinical summaries, and other genetic findings.

6.5 Summary and Discussion

Following a comprehensive search strategy: (i) employing 2 gene panels, one for known epilepsy genes, one for previous reported gene findings in EASD; (ii) performing individual familial triome analysis with *de novo*, recessive, X-linked recessive models of inheritance; and (iii) looking for genes with inherited variants in 3 (5%) or more families, a total of 82 variants were identified in 59 genes.

Only 13 genes had variants identified in more than 1 family, and only 6 had variants identified in 3 or more families, confirming genetic heterogeneity in LKS.

All the gene variants identified for each LKS proband/family have been presented in the **Appendix** alongside each proband's clinical summary. This is so that the relevance of each genetic variant can be considered after taking into account each proband's clinical features and other genetic findings.

14 of the 54 probands (12/38 probands with classical LKS, and 2/16 with atypical symptoms) did not have any genetic findings. 26 probands (15 with classical LKS and 11 with atypical symptoms) had more than one genetic finding which may have summative contributions to their phenotypes (**Appendix- Table 8-1).**

Review of each proband's clinical features and their genetic findings revealed likely alternative diagnoses for 2 probands with atypical symptoms for LKS: childhood apraxia of speech (CAS) for Case 86 (**Chapter 5, Section 5.5.3.1**), and Wiedemann-Steiner Syndrome (WDSS) for Case 58 (**Section 6.2.3.3**).

Of the 59 genes identified, 16 have been classified as pathogenic/likely pathogenic by ACMG criteria. However, even for genes carrying only variants of uncertain significance, most have interesting neurological functions that suggest they may have, at least, a contributory role in the pathogenesis of LKS.

Overall, of the 59 genes identified, 51 (86.4%) are known to have neurological function (detailed in the **Appendix,** the sections above and summarised in **Table 6-16**). The majority, 43/59 (72.8%) have functions that have established links to long term potentiation (LTP) or seizure mechanisms. These are involved in the regulation of excitatory/inhibitory neural activity and processes essential for normal cognitive

development. Another 8 genes have neurological function but no study has, to date, demonstrated direct links to LTP/seizure mechanisms. Some genes, such as *DOCK8* and *ADGRV1,* and *PHF20* have been proposed to have links to dendritic outgrowth or LTP mechanisms based on their known interaction with certain other proteins or on the known functions of closely related members of their gene family. However, these theories have not been substantiated by functional investigations (**Table 6-16**).

In **Table 6-17,** I have distinguished my favourite candidate genes by ranking all the candidates identified in this study according to: (i) top candidates- genes with neurological function and pathogenic/likely pathogenic variants in classical LKS probands; (ii) second- genes with neurological function and pathogenic/likely pathogenic variants in probands with symptoms atypical for LKS; (iii) third- genes with neurological function and variants of uncertain significance identified in 2 or more families; (iv), fourth- genes with neurological function and variants of uncertain significance identified in 1 proband with classical LKS, (v) fifth- genes with neurological function and variants of uncertain significance identified in 1 proband with symptoms atypical for LKS and lastly, (vi) genes with uncertain function in the CNS.

In conclusion, lessons I have gained from genetic analysis of this LKS cohort include: (i) LKS is likely to be genetically heterogeneous; (ii) in this phenotype there is an enrichment of gene variants involved in LTP/epileptogenesis pathways; which may offer some insight into LKS pathogenesis, and (iii) top novel candidate genes for this phenotype include *NPRL3, TRPC1, GABBR2, SCN1A, ERRFI1, CTXN3 and IRX6.*

Table 6-16: Summary of function of genes identified in WGS/WES analysis

GENES WITH NEUROLOGICAL FUNCTION ASSOCIATED WITH LTP/SEIZURE MECHANISMS				
Channel genes mediating neuronal excitation or inhibition				
Gene	**Function**	**Case(s) identified in /Inheritance**	**Analysis identified in**	**ACMG classification**
TRPC1	A tyrosine-kinase receptor activated Ca^{2+} permeable non-selective cation channel implicated in several neuronal functions including neuronal excitability, excito-toxicity and neurite outgrowth	46; *de novo*	Individual triome *de novo*	LP
SCN1A	The alpha subunit of a voltage-gated sodium channel, that is crucial for regulating sodium influx for the creation of action potentials	63, *de novo*	Epilepsy panel	LP
GABBR2	Subunit 2 of the $GABA_B$ receptor which mediates slow synaptic inhibition within the brain	89, *de novo*	Epilepsy panel	Pathogenic
CACNA2D1	The alpha-2/delta subunit of voltage-dependent Ca channels. This protein has a role in the trafficking of voltage-gated Ca channels and has a critical role in synaptogenesis including the recruitment and stabilization of NMDA receptors on the post-synaptic membrane[3]	8, UF	Epilepsy panel	VUS
KCNQ3	Part of a family of voltage gated potassium channels that generates the neuronal M-current with an important role in regulating neuronal excitability	74, UF	Epilepsy panel	VUS
GRIN2D	N2D subunit of NMDA receptor a receptor with a role in excitatory neurotransmission and LTP	90*, UM	Epilepsy panel	VUS
GABRB1	The beta-1 subunit of the $GABA_A$ receptor, a pentameric channel that mediates fast inhibitory synaptic transmission in the CNS[5]	64*, UM	Epilepsy panel	VUS
Cellular signalling/metabolic pathways for dendritic outgrowth and synaptogenesis				
Gene	**Function**	**Case(s) identified in /Inheritance**	**Analysis identified in**	**ACMG classification**
NRPL3	Nitrogen permease regulator 3 like protein, part of the GATOR1 (Gap activity towards Rags) complex that serves as a negative regulator of the mammalian target of rapamycin (MTOR) pathway which in turn has many functions including cell proliferation, motility, apoptosis and synaptic plasticity	45, AS, UM 72, UF	Epilepsy panel	LP VUS

Gene	Function	Case(s) identified in /Inheritance	Analysis identified in	ACMG classification
PLEKHG2	A Rho- guanidine exchange factor (GEF) with a role in actin cytoskeleton arrangement, neuronal network formation, dendritic arborization and spine formation	43*, BP	Individual triome recessive	VUS
ARHGEF4	A Rho- GEF, that inhibits synaptic localization of post-synaptic density- 95 (PSD-95), a major scaffolding protein in the post-synaptic density. Through acting as a specific guanine-exchange factor, for the GTPase CDC-42 (cell-division cycle-42), ARHGEF4 has also been found to stimulate dendritic outgrowth	44, UM 30*, UF	EASD panel	VUS
ARFGEF2	An ADP-ribosylation factor GEF. Through activation of ADP-ribosylation factors 1, it regulates neuronal migration and through interaction with RhoA regulates Golgi polarization and dendrite morphogenesis in hippocampal neuronal cells	7, UF	EASD panel	VUS
ERRFI1	A cytoplasmic protein that is induced by the MAPK/ERK signalling pathway during LTP. Evidence suggests this protein is involved in regulation of neurite outgrowth and cortical neuron migration	53, *de novo*	Individual triome *de novo*	LP
PDE4D	Phosphodiesterase enzyme 4D, which hydrolyses cyclic adenosine- monophosphate (cAMP), a key intracellular signalling molecule. Regulates cAMP/PKA/CREB signalling in the brain.	70, UM	EASD panel	VUS
SOCS7	Inhibits cytokine signalling by linking signalling molecules to E3-ubiquitin ligases. SOCS7 interferes with the reelin signalling pathway, a pathway important for postnatal dendritic growth and synaptic plasticity, through degradation of one of its effector proteins DAB1 (disabled-1).	43*, *de novo*	Individual triome *de novo*	LP
DIP2B	Disco interacting protein 2, a protein which contains a crotonobetaine/carnitine CoA ligase domain related to acyl-CoA synthetases. Demonstrated to be important for axonal development and synaptic transmission. May be involved in the synthesis of acyl-CoA and acetylation of alpha-tubulin, critical for stabilization of microtubules and axonal outgrowth.	86*, APS	EASD panel	VUS
RYR3	Ryanodine receptor type 3 with a role for releasing calcium from intracellular stores in the ER. This calcium release has an important role to play in synaptic plasticity and memory formation.	64*, UM	Epilepsy panel	VUS
EPHB2	A tyrosine kinase receptor with roles in the function and plasticity of excitatory synapses. EPHB2 mediates memory recall in the auditory cortex and hippocampus	64*, UM	EASD panel	VUS
NOTCH1	A single-pass transmembrane receptor protein with a role in cell-proliferative signalling in neurogenesis. NOTCH signalling may also have a role in NMDA receptor downstream synaptic potentiation and epileptogenesis, through dendritic spinogenesis or facilitation of synaptic vesicle neurotransmitter release	7, UM 8, UF 53, UM	3 or more families AD analysis	VUS

Gene	Function	Case(s) identified in /Inheritance	Analysis identified in	ACMG classification
ABCA7	ATP (adenosine triphosphate)-binding cassette sub-family A member 7. This protein is involved in the regulation of amyloid precursor protein (APP) processing and in the phagocytosis of amyloid-beta aggregates (ABA). Both APP and ABA are involved in many processes for LTP, including NMDA receptor subunit expression, neurite outgrowth, synapse formation and neurotransmitter release.	61, BP	Individual triome recessive	VUS
CTXN3	Cortexin-3 may be involved in the development of GABA-ergic neurotransmission during CNS development. CTXN3 has also been implicated in the metabolism of APP which has several roles in LTP as mentioned above for *ABCA7* function.	48, *de novo*	Individual triome *de novo*	LP
Synaptic membrane associating proteins with roles in cytoskeletal structure, vesicular trafficking, receptor re-cycling, neurotransmitter release, axonal guidance and/or autophagy				
Gene	**Function**	**Case(s) identified in /Inheritance**	**Analysis identified in**	**ACMG classification**
WDFY3	Through its role in autophagy and may have an important role in axon guidance, establishing neuronal connections and neuronal migration	30*, *de novo*	Individual triome *de novo*	Pathogenic
GRIP1	Glutamate receptor-interacting protein 1, a synaptic scaffold protein with a well-established role in the stabilization and recycling of AMPA receptor subunits	60, UM	EASD panel	VUS
BSN	A component of the pre-synaptic cytomatrix, with a role in cytomatrix organization for neurotransmitter release, maturation of glutamatergic synapses and neuroplasticity	64*, UM	EASD panel	VUS
SLC9A9	A Na^+/H^+ Exchanger protein that is located on the membranes of late recycling endosomes. Its role includes the recycling and degradation of neurotransmitter receptors and transporters	84*, UM	EASD panel	VUS
MYH7B	Encodes myosin heavy chain 7B, an actin binding protein that has been shown to have a role in maintaining excitatory synaptic function by regulating dendritic spine structure and AMPA receptor trafficking in the hippocampus	62, APS 16*, APS 64*, AUM 8, UM	3 or more families AD analysis	VUS
CDH9	Encodes a type II classical cadherin one of a family of integral membrane proteins that mediate calcium-dependent cell-cell adhesion. CDH9 regulates synapse development in the hippocampus and has an integral role in establishing neuronal connections	26, UM	EASD panel	VUS
CTNNA3	An actin binding protein with an important role in catenin-cadherin cell-cell adhesion. This protein is involved in stabilising dendritic spines. This protein has also been shown to play important roles in maintaining cell-junctions in neuronal structures and cell-signalling within the cerebellum, functions which have been implicated in speech and language development and autism.	34, UM 46, UM 48, UM	EASD panel	VUS

Gene	Function	Case(s) identified in /Inheritance	Analysis identified in	ACMG classification
DIAPH3	Encodes a protein that functions in the assembly of actin filaments. DIAPH3 has important roles in axonal guidance, neuronal migration and neurite formation in the developing brain. Inhibition of DIAPH3 pathways in murine hippocampal neurons result in reduced synaptic activity	73, UF	EASD panel	VUS
VPS13A	Encodes chorein, a protein which binds to phosphatidylinositol lipids on cell membranes and interacts with cytoskeletal proteins. Its many functions including maintenance of cytoskeletal architecture, vesicular trafficking, neurotransmitter release, autophagy, neuronal cell survival and mitochondrial function	50*, BP	Individual triome, recessive	VUS
LAMA5	Encodes laminin alpha-5, an extracellular matrix glycoprotein with a role in the regulation of dendritic spine density and synaptic stability in the hippocampus	86*, APS 50*, UM 81*, UF	3 or more families AD analysis	VUS
LGALS3	Encodes lectin, galactoside soluble protein-3, a member of the lectin and beta-galactoside binding family of proteins, with roles including cell-cell adhesion, cell-matrix interaction and macrophage activation. A study has reported elevated serum levels of this protein in refractory epilepsy compared to control. This gene is also upregulated in a pilocarpine induced TLE murine models	78, UM	EASD panel	VUS
Gene transcription/Epigenetics				
Gene	**Function**	**Case(s) identified in /Inheritance**	**Analysis identified in**	**ACMG classification**
CHD2	Encodes chromodomain helicase DNA-binding protein 2, an adenosine triphosphate dependent chromatin re-modeler which regulates gene transcription. Demonstrated to have important functions in synaptogenesis and regulation of neuronal excitability.	44, UM	Epilepsy panel	VUS
RRN3	RNA polymerase 1 Transcription factor 1A - activates RNA polymerase 1 which transcribes nucleolar rRNA genes important for proliferative growth including that of neurite- extending neurons. Implicated in neural plasticity and epileptogenesis	55, NPS	EASD panel	VUS
RORB	Encodes retinoid related orphan receptor B, a ligand dependent transcription factor. Its roles in the CNS include transcriptional control of neuronal differentiation, neurogenesis and thalamocortical axon guidance	57* *de novo*	Individual triome, *de novo*	LP
SETD5	Encodes SET [Su(var)3-9, enhancer of zeste, trithorax] domain containing protein 5. It has been demonstrated that the epigenetic processes mediated by SETD5 regulate neural stem cell proliferation, synapse formation and glutamatergic transmission	75*, UF 86*, APS	Epilepsy panel	VUS

Gene	Function	Case(s) identified in /Inheritance	Analysis identified in	ACMG classification
KMT2A	A histone methyltransferase enzyme that has a key role in gene expression regulation during development. Within the CNS, it has been shown that genes regulated by KMT2A are involved in key pathways for neurogenesis, synaptic development and neuroplasticity	58*, *de novo*	Individual triome *de novo*	LP
FOXP2	Forkhead box protein P2, a member of a family of transcription factors. FOXP2 is thought to coordinate pathways important for brain development and function, including synaptogenesis	86*, APS 75*, UM	EASD panel	Pathogenic VUS
RBFOX1	RNA- binding FOX1 Homolog 1, an RNA binding protein that regulates alternative splicing of neuronal transcripts. This protein regulates the expression of an isoform of brain derived neurotrophic factor (BDNF) tyrosine kinase receptor. RBFOX1 also has important roles in cortical development, neuronal maturation, inhibitory synaptic transmission and excitatory synapse downscaling	84*, UM	EASD panel	VUS
MBD5	Encodes methyl-CpG-binding domain (MBD) protein 5. Like other MBD proteins, MBD5, has a role in transcriptional activation. It has also been found to have a role in neurite outgrowth and neuronal differentiation	90*, UM	Epilepsy panel	VUS
PAX6	Encodes paired box gene 6, a transcription factor integral to the development of many tissues including the brain. *PAX6* haploinsufficiency has been shown to lead to impaired hippocampus synaptic plasticity	89, APS	EASD panel	VUS
Inflammation/neuronal apoptosis/epileptogenesis				
BIRC6	A ubiquitously expressed protein, with a role in the control of apoptosis. In the brain, BIRC6 has been found to be neuro-protective. Downregulation of this protein resulted in reduced neuronal viability and increased susceptibility of neurons to excitotoxicity	63, APS	EASD panel	VUS
LTBP1	Encodes latent transforming growth factor β (TGF- β)-binding protein-1, which regulates the function of latent TGF- β. A study looking at the transcriptomic profiles of a murine model of TLE has identified *LTBP1* as a key regulator in epileptogenesis	74, UF	EASD panel	VUS
NLRP3	Part of an inflammasome complex that stimulates the release of inflammatory factors like Il-1B. The NLRP3 inflammasome has been implicated in epileptogenesis, and epileptic neuronal apoptosis	30*, *de novo*	Individual triome, *de novo*	VUS

GENES WITH NEUROLOGICAL FUNCTION WITHOUT ESTABLISHED DIRECT LINKS TO LTP/SEIZURE MECHANISMS				
Cell- signalling/metabolic pathways				
DOCK8	A GEF that activates the Rho-GTPase, Cdc42. This protein regulates dendritic cell migration and is known to have an important role in the immune system. DOCK8 has also been shown to have a role in neuro-inflammation through mediating microglial migration and phagocytosis. It has also been suggested though not demonstrated that DOCK8 may have a role in polarized axon growth through its interaction with CDC42 like other members of the DOCKC group DOCK6 and DOCK7.	3, AC 44, UM 74, UM	3 or more families AD analysis	VUS
ADGRV1	A member of the adhesion family of G-protein coupled-receptors. Some members of this family – ADGRL1 have a role in axon guidance and synaptogenesis. ADGRV1 is found at synapses at cochlear and retinal cells and has been found to be needed for GABA-ergic interneuron. development in the auditory cortex. It remains to be established if it may have a more widespread role in the rest of the CNS.	29, UF 52*, UF 78, UF	Epilepsy panel	VUS
Roles in cytoskeletal structure				
COL4A2	Encodes the α2 chain of type IV collagen, a structural component of basement membranes in several organs in the body, including the brain.	41, *de novo*	Individual triome, *de novo*	VUS
HSPG2	Heparan sulphate proteoglycan 2, aka perlecan, a multi-functional protein with a role in the maintenance of extracellular matrices and various signalling pathways. It is believed to be involved in brain development, as perlecan-null mice have exencephaly but the mechanisms for this are not well understood.	66, UM	EASD panel	VUS
Gene transcription/epigenetics				
RBM15	Encodes RNA-binding motif protein 15, a key regulator of RNA methylation, with important roles in many cellular processes including haematopoietic cell homeostasis. It has also been implicated in the processes of neurulation and neural morphogenesis in early brain development	81*, *de novo*	Individual triome, *de novo*	LP
PHF20	Encodes plant homeodomain finger 20, a protein with roles in transcriptional regulation and DNA repair. It has been suggested though not proven that by associating with the H3K4- specific methyltransferase, MLL1, *PHF20* may be implicated in the regulation of genes that are involved in neurogenesis, and synaptic development pathways.	57*, *de novo*	Individual triome, *de novo*	VUS
SMC1A	Encodes structural maintenance of chromosomes 1A protein, a subunit of the cohesin-core complex that tethers sister chromatids together to facilitate smooth segregation during cell division. This protein has a role in gene transcription regulation and DNA damage repair.	65, UM (X-linked)	Epilepsy panel	VUS
IRX6	A homeobox gene encoding a transcription factor. The function of this gene is not yet well established. However, IRX-6 has been found to be involved in neurogenesis in murine models	82, *de novo*	Individual triome, *de novo*	LP

GENES WITH UNCLEAR CNS FUNCTION				
IQCA1	A component of the nexin-dynein regulatory complex, which has a role in the maintenance of the distal axoneme and the regulation of ciliary motility. Its role in the CNS is not well characterised.[1]	8, *de novo*	Individual triome, *de novo*	LP
LIMD1	Encodes LIM-domain containing protein 1, a transcription regulator. It is involved in the regulation of cell adhesion, cytoskeletal organisation, cell morphology and cell migration. Its function in the CNS is not well characterised.	57*, *de novo*	Individual triome, *de novo*	LP
MYSM1	Encodes an enzyme with deubiquitinase catalytic activity. MYSM1 regulates gene regulation through deubiquitination of histone-2A and non-catalytic contacts with other transcriptional regulators. Its role is best characterized as an important regulator of haematopoiesis and immunity. Its role in the CNS has not yet been well-established	82, *de novo*	Individual triome, *de novo*	VUS
PYGL	Encodes glycogen phosphorylase, responsible for glycogen degradation. Its role in the CNS is not clear	61, *de novo* 3, AC	Individual triome, *de novo*	VUS
CSPP1	Encodes centrosome spindle pole associated protein 1, a core centrosomal protein that has a role in the regulation of ciliary axoneme formation. It is implicated in Hedgehog signalling pathways important for cell-differentiation.	86*, APS 24, UF	EASD panel	VUS
ZNF646	Encodes zinc finger protein 646. The function of this protein is unclear as it has not been well studied. As a zinc finger protein, it is possible, it has a role in transcriptional regulation or DNA repair[3]	41, UF	EASD panel	VUS
TCTEX1D2,	*encodes* a dynein light chain, a protein required for intra-flagellar transport important for ciliary growth and signalling	66, UM	EASD panel	VUS
ADAMTSL4	Encodes A-Disintegrin and Metalloproteinase with Thrombospondin Motif Like- Protein Its function is not completely understood but it is related to fibrillin-1 and is likely to have a role in extracellular matrix homeostasis	52*, BP	Individual triome, recessive	VUS

AC: affected child; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APS: awaiting parental Sanger sequencing; AUM: absent in unaffected mother; BP: bi-parental; CNS: central nervous system; CREB: cyclic-adeno-monophosphate response element binding protein; DNA: deoxyribonucleic acid; EASD: epilepsy aphasia spectrum disorder; GABA: Gamma-amino-butyric acid; LP: likely pathogenic; NMDA: N-methyl- D-aspartate; PKA: protein kinase A; RNA: ribonucleic acid; TLE: temporal lobe epilepsy; UF : inherited from unaffected father; UM: inherited from unaffected mother; VUS: variant of uncertain significance;

Table 6-17 Ranking of best candidate genes

Top Candidates: Genes with Neurological Function And Pathogenic/Likely Pathogenic Variants In Probands With Classical LKS	*NPRL3*, TRPC1, GABBR2, SCN1A, ERRFI1 CTXN3, IRX6,*
Genes with Neurological Function And Pathogenic/Likely Pathogenic Variants In Probands With Atypical Symptoms For LKS	*WDFY3, RORB, SOCS7, KMT2A, NLRP3, RBM15, FOXP2**
Genes with Neurological Function And Variants Of Uncertain Significance In More Than 1 Family:-	*MYH7B, ADGRV1, CTNNA3, DOCK8, NOTCH1, LAMA5, ARHGEF4, SETD5, LTBP1*
Genes with Neurological Function And Variants Of Uncertain Significance In 1 Proband With Classical LKS:-	*CACNA2D1, CHD2, KCNQ3, CDH9, DIAPH3, PAX6, ARFGEF2, HSPG2, BIRC6, RRN3, LGALS3, PDE4D, COL4A2, ABCA7, SMC1A*
Genes with Neurological Function And Variants Of Uncertain Significance In 1 Proband With Atypical Symptoms For LKS:-	*RYR3, GRIN2D, GABRB1, MBD5, BSN, EPHB2, DIP2B, GRIP1, RBFOX1, SLC9A9, PHF20, PLEKHG2, VPS13A,*
Genes With No Clear Neurological Function:	*PYGL, IQCA1, LIMD1, MYSM1, CSPP1, ZNF646, TCTEX1D2, ADAMTSL4*

* Has a likely pathogenic variant in 1 family and a variant of uncertain significance in 1 other family.

7 Chapter 7: Conclusions and future directions

7.1 Insights, limitations and future directions

As I have discussed in the Introduction (**Chapter 1**), epilepsy is defined not just by clinical seizures but also by its associated co-morbidities, namely the neurobiological, cognitive, psychological and social consequences of this condition. From having undertaken this PhD, it is clear that Landau Kleffner syndrome (LKS) is a classic example of how this definition rings true.

One fundamental question this research study has aimed to answer relates to the underlying mechanisms governing neurocognition and epilepsy pathogenesis in LKS. Through the detailed clinical and genetic work- up of this extensive LKS cohort, we have gained several precious clues, although there remains much more to be understood.

This concluding chapter: (i) summarizes the insights we have gleaned from both the clinical and genetic aspects of this study, (ii) ponders on what remains to be understood, (iii) considers the limitations of this study, and (iv) contemplates future directions for research.

7.2 Mechanisms governing neurocognitive deficits in LKS- insights gained

7.2.1 Insights gained from study of the clinical cohort

Chapter 3 examined in detail the clinical characteristics of this extensive LKS cohort. This phenotyping study confirmed that significant neuropsychological co-morbidities occur in LKS. In addition to universal speech and language impairment by definition, our patients also had a number of other disease-related features, including behavioural issues, impairment in non-verbal IQ, and motor difficulties, that significantly impacted on their quality of life (**Chapter 3, Section 3.3**).

The concept that epilepsy syndromes carry both seizure and non-seizure related disease burden is increasingly recognised in both the epilepsy field and epilepsy classification systems. In Chapter 1, the new 2017 ILAE definitions for “epileptic encephalopathy”, “developmental encephalopathy” and “developmental and epileptic encephalopathy”

were introduced, in part to account for these observations. These terms were designed to try and distinguish the impact of developmental processes versus epileptic processes on cognitive burden. Through clinical delineation of this cohort, I have considered the question: "are the neuropsychological deficits encountered in LKS more likely to be the result of just epileptic processes, or part of a broader developmental or genetic process?"

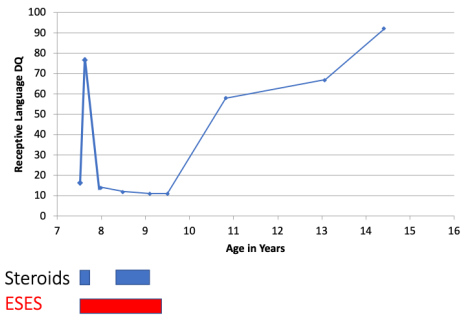
Today, electrical status epilepticus in slow wave sleep (ESES) continues to be poorly understood. Indeed, the definition of ESES continues to be debated. The original definition by Patry et al in 1971 was sleep activation in slow-wave sleep to the extent that spike-wave discharges occupy more than 85% of slow-wave sleep time, i.e. a spike-wave index (SWI) of > 85% (Patry et al., 1971). This present study has kept to this original definition. However, since Patry et al's paper in 1971, many different SWI thresholds have been used to define ESES ranging from 25% to 90% (Scheltens-de Boer, 2009). In addition to SWI, many feel that the concomitant presence of neuropsychological impairment is a necessary criterion for the diagnosis of ESES (Tassinari and Rubboli, 2019). However, others (as many as 60% of American paediatric neurologists) do not consider demonstration of developmental regression mandatory for ESES diagnosis (Fernández et al., 2013).

The observation that cognitive/language/behavioural impairment can occur at a range of different SWIs suggest that an SWI threshold of 85% may not be important for the definition of ESES.

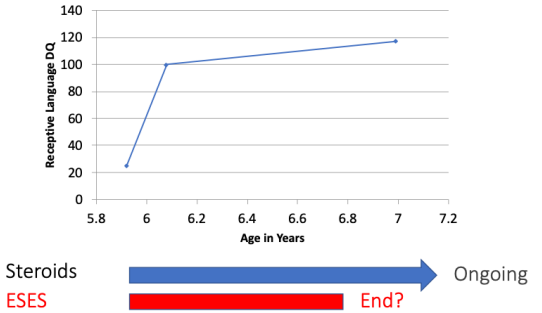
A study by van den Munckof et al in 2016, reported that in children with ESES (defined by a SWI of at least 50%), subjective cognitive improvement, as reported by parents was strongly associated with SWI decrease. However, cognitive assessments do not reflect a significant increase in IQ (van den Munckhof et al., 2018). The observation that that there is not a linear correlation between the severity of neuropsychological deficits and SWI have led some authors to suggest that other parameters such as impairment of synaptic homeostasis as reflected by slow wave slope may be important in unveiling the mechanisms leading to the encephalopathy patients experience (Bölsterli Heinzle et al., 2014, Bölsterli et al., 2017).

In this study, I found no significant correlation between either clinical seizures or the presence of ESES with language outcomes or outcomes in adulthood (**Chapter 3; *Table 3-2* and Table 3-3**). In the individual profiling for each patient, we also noted that there were patients for whom speech and language regression *preceded* the onset of seizures and EEG abnormalities (**Appendix**). There was inconsistent correlation between language skills and the severity of clinical seizures, the presence of ESES, or the use of steroid therapy; with some individuals regaining language skills despite ongoing ESES (***Figure 7-1***).

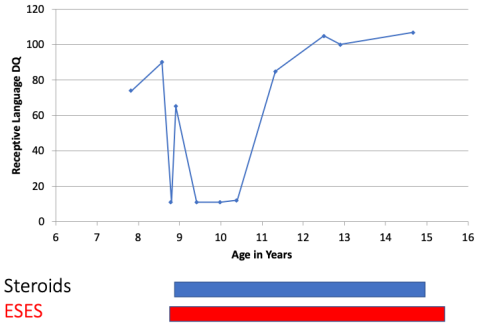
Figure 7-1 Profile of individual patients – correlating receptive language developmental quotient (DQ) with timing of ESES and steroid therapy



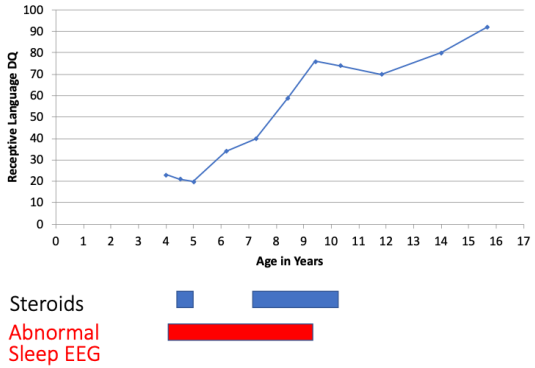
Patient 48: improvement on steroids despite ESES, then relapse



Patient 61: remarkable improvement and maintenance despite ESES



Patient 34: improvement and deterioration not correlating with steroids or ESES



Patient 35: Improvement despite abnormal sleep EEG with activation in slow wave sleep

Data obtained in collaboration with Dr Maria Clark and Dr Rebecca Greenaway, Developmental Epilepsy Clinic, Great Ormond Street Hospital

Before the onset of my PhD study, a separate study was carried out within the Developmental Epilepsy Clinic at Great Ormond Street Hospital, examining the relationship between neuropsychological co-morbidities and EEG findings/steroid therapy (unpublished personal communication, Dr Maria Clark and Dr Rebecca Greenaway). In this study, longitudinal data for verbal expressive developmental quotient (DQ), verbal receptive DQ and non-verbal DQ was collected for 49 patients presenting with LKS. This study then used generalised estimating equations to correlate this data with age at assessment, EEG findings and steroid therapy. Briefly, the conclusions from this study were that the most important predictors for expressive and receptive language skills were patient age at the time of assessment and whether or not the child was on steroids. EEG was not found to be a significant predictor although there was a trend for it to be. There were no significant predictors for non-verbal DQ. Data from this currently unpublished study also suggested that there was no significant effect of steroid therapy on EEG, with each having independent effects on language outcome.

Overall, these clinical observations suggest that whilst epileptiform activity may contribute to cognitive deficits in LKS, LKS is unlikely to be a pure epileptic encephalopathy. Coupled with the fact that this study, like other studies, has found a consistent age-predilection, and that up to 48% of patients have a positive family history (**Chapter 3; Table 3-1**), I believe that neuro-developmental and genetic factors are likely to have a significant contribution to the neuro-cognitive deficits LKS patients face.

7.2.2 Insights gained from molecular genetic investigations

The discovery that 8-20% of individuals with LKS and related epilepsy aphasia spectrum disorders harbour mutations in the gene *GRIN2A*, further lends support to the role that genetic neurodevelopmental processes have to play in neuro-cognitive deficits in LKS.

Within our cohort, I have discovered 7 *GRIN2A* mutations. 5 of these are protein truncating variants that can be expected to have loss-of-function effects. In Chapter 4, I demonstrated through different functional assays, that the remaining 2 missense mutations similarly have overall loss-of-function effects secondary to reduced cell surface expression of the mutant protein. Our results corroborate with the results of other investigators (Swanger et al., 2016, Sibarov et al., 2017), enabling us to come to

the conclusion that although there are some *GRIN2A* mutations that have gain of function effects, most *GRIN2A* mutations associated with LKS and EAS have loss of function effects (**Chapter 4; Figure 4-8**).

GRIN2A encodes the N2A subunit of the N-methyl- D-aspartate (NMDA) receptor. As illustrated in **Chapter 1, Section 1.2.2,** in addition to mediating excitatory neuro-transmission in the brain, NMDA receptors have key roles in long term potentiation (LTP), an important physiological mechanism involved in learning and memory. It would therefore make sense that long-term loss of NMDAR function due to *GRIN2A* mutations at a critical stage of neurodevelopment would impair the mechanisms of long term potentiation which may contribute to neuro-cognitive impairment in LKS.

Furthermore, it is interesting to note that the majority of the candidate genes I have identified in WES/WGS analysis of this cohort, also impact on LTP (**Chapter 6; Table 6-16**). Future functional investigations may help determine the effect of these identified variants on protein function. Interrogation of other LKS cohorts and discovery of more variants in all the candidate genes we have identified, would further lend support to the role these genes have to play in the pathophysiological mechanisms underpinning LKS.

7.3 What remains to be understood: future perspectives

Whilst this study has contributed to our understanding of LKS and the pathophysiological mechanisms leading to LKS symptomology, much remains to be understood. This section explores some unanswered questions.

7.3.1 Pathophysiological mechanisms for seizures and cognitive dysfunction.

There is much we still do not understand about the pathophysiology behind seizures and cognitive co-morbidities. As mentioned in **Chapter 1, 1.3.1,** there are common causative factors for seizures and cognitive dysfunction. Since the basic pathophysiology of seizures involves an imbalance between excitatory and inhibitory activity in the brain, in addition to its role in learning and memory, the LTP pathway can also be involved in epileptogenesis through increasing neuronal excitability. However,

since LTP is meant to facilitate learning and memory, how does increased neuronal excitability brought about by LTP, then paradoxically lead to cognitive impairment? One possible mechanism is that over-excitation at a synapse leads to neuro-toxicity and subsequently neuronal damage and cognitive impairment (Reisberg et al., 2003).

Following this train of thought, since NMDA receptors mediate excitatory neurotransmission in the brain, one might expect that it ought to be *gain* of function rather than loss of function variants that lead to seizures and cognitive impairment in epileptic encephalopathies like LKS. However, as illustrated in **Chapter 4**; **Figure 4-8**, the majority of *GRIN2A* variants identified in LKS/EASD have been proven to have loss of function effects.

Conversely, it is also interesting to note that the mechanisms underlying NMDA receptor encephalitis involve a reversible reduction in NMDA receptor surface density due to cross-linking of the receptors by auto-antibodies and receptor internalization. This leads to NMDA receptor hypofunction, yet patients present not just with neuropsychiatric clinical features but also with refractory epileptic seizures.

There are a few theories regarding how loss of function *GRIN2A* mutations may bring about seizures. Firstly, some have proposed that loss of function *GRIN2A* mutations lead to upregulation of other NMDA receptor subunits, such as N2B (Balu and Coyle, 2011). The different NMDA receptor subunits have distinct pharmacological properties, and upregulation of N2B in place of N2A i*n vivo* may actually result in overall gain of function effects of NMDA receptor function within the neural network. Secondly, there are many different aspects of NMDA receptor function that may be modified by a single gene variant. It has been demonstrated that a single variant can have both gain of function effects on some aspects of NMDA receptor function and loss of function effects on other aspects. For example, a single variant may have gain of function effects through increased agonist potency, yet have loss of function effects through the promotion of allosteric inhibition (Sibarov et al., 2017). This leads me to postulate that different NMDA receptor functions may have specific roles to play in excitatory neuro-transmission and long-term potentiation and thus, gene disturbance may result in both seizures and deficits in long-term potentiation through differential effects on gene function. Thirdly, it is important to note that expression of NMDA receptors is not

limited to excitatory neurons, they are also expressed on GABAergic interneurons, with the majority being immune-positive for parvalbumin and/or somatostatin. In *SCN1A* Dravet Syndrome, *SCN1A* loss of function variants lead to epilepsy via their effect on inhibitory interneurons, leading to overall neural excitation (Escayg and Goldin, 2010). It is possible that loss of function of excitatory NMDA receptors on inhibitory interneurons would similarly predispose to seizures. Finally, my personal theory is one extrapolated from Alzheimer's disease (Tsai, 2016)- it may be possible that NMDA receptor hypofunction may lead to over-activation of secondary compensatory mechanisms that result in seizures and/or neurotoxicity.

The question of which theory is most accurate is important because it impacts on therapeutic options. This is further discussed in **Section 7.3.4**.

7.3.2 LKS - an oligogenic or multi-factorial disorder?

It is difficult to directly extrapolate from *GRIN2A* gene variants to LKS symptomology when there is such wide variation in the symptoms that *GRIN2A* variants cause. As mentioned in **Chapter 4, Section 4.3**, the same *GRIN2A* variant within a family can result in a wide variety of phenotypes – from some individuals being completely asymptomatic, to others just having mild speech and language difficulties, to those with isolated speech and language regression with seizures (LKS) to patients with global developmental skill regression with seizures (ECSWS). *GRIN2A* is widely expressed in the brain and it is difficult to explain why any given mutation can cause such a wide spectrum of presentations leading to both inter- and intra-familial variability.

The fact that *GRIN2A* accounts for only a minority of patients with LKS, together with the observed phenotypic variability and incomplete disease penetrance, breed the postulation that the path to LKS is multifactorial and may require more than just the presence of a *GRIN2A* mutation in an affected individual. It is indeed possible that *GRIN2A* variants lead to a genetic predisposition to LKS through the mechanisms discussed previously, but perhaps a "second hit" is required before clinical features of LKS manifest. This "second hit" might take the form of other genetic modifiers or environmental factors.

Within the GOSH LKS cohort, 3/7 (42.8%) *GRIN2A* mutations identified were inherited, and of these 2/3 (66%) were inherited from unaffected parents. These percentages are consistent with previous literature reports of *GRIN2A* mutations within EASD (**Chapter 4, Table 4-2**). For LKS family 11 (**Appendix**) the proband inherited a *GRIN2A* p.Arg518His mutation from an asymptomatic father and had a similarly affected brother with LKS. For family 49, the proband inherited a p.Val430Glufs*18 *GRIN2A* mutation from an unaffected mother, and had a brother with childhood epilepsy with centrotemporal spikes (**Appendix**). DNA was unfortunately not available for both affected siblings but given their symptoms, it is possible that they may have inherited the same *GRIN2A* mutation as their genetically-confirmed sibling. What could it be then that makes two siblings within a family, presumably with the same mutation, manifest symptoms when the parent from whom they inherited the variant remains asymptomatic?

A possible explanation could be that the phenotypes of patients with different clinical presentations are modified by other genetic variants (possibly occurring *de novo* or inherited from the other parent). As such, it may be possible that LKS is not a monogenic disorder, but rather ***an oligogenic or polygenic disorder*** in which the combined effect of a number of genetic variants (together with currently undetermined epigenetic and environmental factors) is required before symptoms manifest. In this LKS cohort, 26/54 probands have more than 1 genetic finding **(Appendix, Table 8-1).** It is possible that their different genetic findings may have synergistic effects on their phenotype. For example, Case 74 and Case 78 both only have inherited variants classified as variants of uncertain significance (**Appendix**). However, some of their genes were paternally inherited and others were maternally inherited. All of these genes are associated with important neurological functions and these variants are all predicted to be pathogenic by in-silico prediction algorithms. It is possible that each parent, having only one variant remains asymptomatic, however, the combination of two deleterious gene variants leads to symptoms in the affected child. Case 64 is another example, with multiple variants of uncertain significance that may all have some contributory effect on his symptoms.

With regard to environmental factors, it is possible that siblings are raised in similar environments which are distinct from those of their unaffected obligate carrier parents. As mentioned in **Chapter 4, Section 4.3.5**, there have been reports of LKS symptoms

occurring after minor head injury or after a viral infection (Bhardwaj et al., 2009) including some patients from Landau and Kleffner's original paper (**Chapter 1, Table 1-2**). Some patients in our LKS cohort also reported a similar experience (**Appendix-Cases 8, 29, 79**). It is possible therefore that environmental factors can also provide a "second hit" for the development of LKS in genetically predisposed individuals.

7.3.3 Role of immune dysfunction in LKS

Whilst considering other aetiological factors for *GRIN2A* negative LKS patients, it may be important to also re-visit the possibility that LKS may be an autoimmune/immune mediated disorder.

One of the patients who was referred for this LKS study was an 8 year old girl who presented quite classically with seizures, aphasia and challenging behaviour. She screened negative for *GRIN2A* mutations but was later found to be positive for NMDA receptor antibodies. On this basis, she was later excluded from this LKS study. Her EEG showed centrotemporal spikes which activated in sleep, but was also significant for delta brushes. Interestingly, a 5 year old girl (Case 79) presented shortly after, with seizures, aphasia and a movement disorder. This latter patient's EEG also showed centrotemporal spikes which activated in sleep, and was also reported to have delta brushes. This latter patient screened negative for NMDA receptor antibodies but was found to have a *de novo,* previously reported *GRIN2A* mutation.

There are many neurological disorders that can present due to both a genetic and immune aetiology. One classical example is myasthenia gravis, which can be caused either by auto-antibodies or by genetic variations targeting various proteins involved in neuro-transmission at the neuro-muscular junction (Iyadurai, 2020). Furthermore, several genes associated with epilepsy encode proteins that are also targeted in autoimmune encephalitis. Examples include: (i) *CNTNAP2* mutations and limbic encephalitis secondary to contactin associated protein 2 (CASPR2) autoantibodies; (ii) *LGI1* mutations and limbic encephalitis secondary to LGI1 autoantibodies and (iii) mutations in GABA receptor genes and autoimmune encephalitis due to autoantibodies to GABA receptors. There is often significant overlap in the clinical features observed in these genetic epilepsies and autoimmune encephalitides. Additionally, a role for

autoimmune aetiology is supported by the fact that some LKS patients develop symptoms after a viral prodrome, and that patients often respond to corticosteroid therapy. It is therefore conceivable that autoimmunity may cause LKS independently of genetic factors, or it may lead to LKS in genetically predisposed individuals.

7.3.4 Targeted treatments?

7.3.4.1 NMDA receptor agonists?

As discussed in **Chapter 4 Section 4.12,** early functional investigations on a limited number of reported *GRIN2A* variants suggested an overall gain of function effect. As such, memantine, a pharmacological NMDA receptor antagonist which works by blocking the NMDA receptor's ion channel, was proposed to be a viable targeted treatment option. Indeed, a trial of memantine was given to a 6.5 year old boy with early infantile epileptic encephalopathy with a p.L812M *GRIN2A* mutation, a mutation found to have gain of function effects on functional investigation. Although this was reported to result in significant reduction in seizure frequency, it had no significant effect on cognitive function (Pierson et al., 2014).

Latter investigations, including the results of this current study, however, have proved that the majority of *GRIN2A* mutations in LKS and EASD seem to have an overall loss of function effect (**Chapter 4, Figure 4-8**). Might it therefore be viable to alleviate symptoms of LKS/EASD with NMDA receptor agonists?

As mentioned in **Chapter 1, Section 1.2.2.2**, NMDA receptors are heterotetrameric. Most NMDA receptors are made up of 2 N1 subunits and 2 N2 subunits (which may be any combination of N2A, N2B, N2C or N2D subunits). The main agonists of N1 subunits are glycine and D-serine, while the main agonists of N2 subunits are glutamate or aspartate. Activation of the NMDA receptor requires binding of glutamate/aspartate to the N2 subunits and binding of the co-agonists, glycine or D-serine to the N1 subunits. Glycine/D-serine binding modulates the amplitude and time course of the glutamate-elicited NMDA receptor response, through increasing receptor affinity for glutamate, reducing receptor desensitization and promoting receptor turnover through internalization (Shleper et al., 2005, Tsai, 2016).

Excessive stimulation of N2 subunits with glutamate has been associated with neurotoxicity (Tsai, 2016). Hence, it makes sense to examine, instead, the role of N1 subunit co-agonists as means of modulating NMDA receptor function. D-serine in particular, rather than glycine, might be the co-agonist of choice to consider, for a number of reasons. Firstly, synaptic N2A subunit containing NMDA receptors and extra-synaptic N2B containing NMDA receptors have different co-agonists, synaptic N2A subunit containing NMDA receptors have D-serine as their co-agonist, whilst extra-synaptic N2B containing NMDA receptors have glycine (Papouin et al., 2012). Whilst inconclusive, synaptic N2A subunit- containing NMDA receptors are believed to be differentially involved in neuro-protection, while extra-synaptic N2B NMDA receptors are differentially associated with excitotoxicity and neurodegeneration (Tsai, 2016). Secondly, whereas glycine is involved in both excitatory and inhibitory neurotransmission in the brain, D serine is highly expressed in the corticolimbic regions and plays a key role in higher-order cognitive functions (Balu et al., 2014). Lastly, glycine has poor central nervous system bioavailability as it has no specific transporter across the blood-brain barrier (Tsai, 2016).

There has been increasing evidence to show that potentiation of the NMDA receptor by the co-agonist, D-serine, may be effective in several neuro-psychiatric disorders including depression, schizophrenia, autism and Alzheimer's disease (Tsai, 2016). In the late phase of Alzheimer's disease (AD), excessive glutamatergic neurotransmission mediated by NMDA receptors are thought to lead to neurotoxicity. Based on this theory, the NMDA receptor antagonist, memantine has been approved as an anti-dementia treatment for moderate to severe AD (Reisberg et al., 2003). However, memantine has limited efficacy in AD particularly in the early phase. This has led some to postulate if glutamate induced neurotoxicity may be a secondary phenomenon related to over-activation of compensatory mechanisms to earlier NMDA receptor hypo-function. NMDA receptor antagonists such as ketamine have been shown to impair spatial learning and verbal skills in healthy humans, and other antagonists such as MK-801 have also been found to induce apoptosis and neurodegeneration both in vitro and in vivo (Yoon et al., 2003). It thus may not be wise to administer NMDA receptor antagonists that worsen neurocognition, particularly early in the disease process.

Studies have found that both natural aging and Alzheimer's disease are associated with decreased density of NMDA receptors and lower D-serine levels (Hashimoto et al., 2004) and that D-serine treatment has been found to have neuroprotective effects (Esposito et al., 2012).

D-cycloserine, is approved for use as an anti-tuberculotic medication (Li et al., 2019). It is on the World Health Organization's List of Essential Medicines, as one of the safest and most effective medications. As an antibiotic, it works by inhibiting peptidoglycan/cell-wall synthesis (Lambert and Neuhaus, 1972). D-cycloserine is also a partial agonist at the co-agonist site of the NMDA receptor. In vivo, it has also been found to raise D-serine levels (Horio et al., 2013).

Administration of D-cycloserine has been found to improve the scores of AD patients on the cognitive sub-scale of the Alzheimer's Disease Assessment Scale (ADAS-cog) (Tsai et al., 1999).

Sodium Benzoate is another safe agent found widely as a food and medication preservative, and as a drug used to treat hyperammonaemia (Snehavardhan et al., 2019). Sodium Benzoate is also an inhibitor of D-amino acid oxidase (DAAO), a flavoenzyme responsible for the degradation of D-serine (Tsai, 2016).

In a 24-week double blind placebo-controlled study, sodium benzoate administration in mild AD patients improved their ADAS-cog score substantially to the range of the normal elderly population, suggesting that DAAO inhibition has the potential to reverse early AD back to normal range (Lin et al., 2014).

Both D-cycloserine and D-serine have also been reported to have positive clinical and electrophysiological effects in NMDA receptor encephalitis (Heresco-Levy et al., 2015). In 2016, D-cycloserine was used as trial therapy for a previously healthy 13 year old female with NMDA receptor encephalitis manifesting generalized seizures, agitation, cognitive deterioration, movement disorder and hypoventilation requiring mechanical ventilation. Her symptoms did not seem to respond to laparoscopic resection of an ovarian teratoma and treatment with methylprednisolone, repeated cycles of intravenous immunoglobulin, cyclophosphamide or mycophenolate mofetil. Five months after presentation, she continued to show no signs of clinical improvement.

Administration of D-cycloserine at this point, resulted in the gradual resolution of seizures, choreic movements and hypoventilation. At follow-up 1 year from symptom onset, the patient was functionally normal with a modified Rankin score of 0 (Guan et al., 2016).

Agents for D-serine potentiation has, to my knowledge, never been tried in LKS. However, recently, L-serine, a precursor of D-serine, was given as dietary supplementation to a child with Rett-syndrome like encephalopathy secondary to an autosomal dominant loss-of-function *GRIN2B* mutation. This treatment was reported to result in significant improvement in neuro-cognitive skills (Soto et al., 2019).

Given that D-serine potentiation has been shown to improve both seizures and neuro-cognition in the face of NMDA receptor hypo-function, it may well have the potential to alleviate symptoms in LKS, at least in those with loss of function *GRIN2A* mutations. As it is possible that other aetiological factors may also converge on the LTP pathway, it may even be possible that augmentation of the LTP pathway through NMDA receptor agonism, may improve symptoms even for patients with LKS due to other aetiologies.

7.3.4.2 Pharmaco-chaperones?

The investigations I performed on the missense variants identified in this cohort demonstrated reduced surface expression of mutant N2A protein. The same has been demonstrated for several other *GRIN2A* variants associated with EASD (**Chapter 4**; **Figure 4-8**). Therefore, a form of therapy to consider might be that of pharmacological chaperones which can act to stabilise a mutant protein and promote its trafficking to the cell surface (Liguori et al., 2020).

To date, I am not aware of any synthetic pharmacological compound which has been developed as a pharmaco-chaperone for NMDA receptor proteins. However, it may be possible to pharmacologically augment the action of endogenous chaperones.

The Sigma-1 receptor (S-1R) is a protein that functions as a chaperone for many ion channels including, sodium, potassium, calcium and transient receptor potential (TRP) channels (of which *TRPC1* is a member). It also binds to and regulates the expression of both N1 and N2 subunits of the NMDA receptor (Morales-Lázaro et al., 2019).

The function of S-1R can be regulated by both endogenous and synthetic ligands. Neurosteroids are its most common endogenous ligands. Dehydroepiandrosterone sulphate (DHEA) and pregnenolone-sulphate act as S-1R agonists and have been shown to positively regulate NMDA receptor trafficking and expression (Morales-Lázaro et al., 2019).

Regulation of NMDA receptors by synthetic ligands of S-1R has also been extensively studied. In murine models, it has been shown that the S-1R agonist, PRE084 reverses learning impairments induced by NMDA receptor antagonists (Maurice et al., 1994). Another S-1R agonist, Cutamesine (SA4503) has also been shown to ameliorate spatial working and memory deficits of rats treated with intra-peritoneal infection of dizocilpine, a competitive antagonist of NMDA receptors (Zou et al., 2000). Lastly, the selective serotonin re-uptake inhibitor (SSRI), fluvoxamine has also been shown to have agonist effects on S-1R. In mice with cognitive deficits induced by NMDA receptor antagonist phencyclidine, fluvoxamine, but not other SSRIs like sertraline or paroxetine, was shown to improve cognitive function. This effect was blocked by S-1R antagonists (Hashimoto, 2015). Furthermore, results from a few clinical studies suggest that treatment with fluvoxamine improved cognitive impairment in patients with schizophrenia (Niitsu et al., 2012).

Pharmacologic S-1R modulation is already being explored as possible therapeutic options in neuropsychiatric disorders, stroke and neurodegeneration (Hashimoto, 2015, Urfer et al., 2014, Penke et al., 2018). Considering that impaired trafficking of N2A subunits is part of pathogenic mechanisms underlying LKS/EASD, S-1R modulation may well also be an efficacious treatment strategy for this group of disorders.

7.3.4.3 Gene therapy

The acceleration in genetic technology since the advent of the Human Genome Project has led to mounting excitement that gene therapy may one day, be able to revolutionize the treatment of disorders with underlying genetic aetiology.

Gene therapy may involve the introduction of a normal replacement allele into target cells to compensate for loss of function gene mutations using viral vectors, or the silencing of a dominant pathogenic mutant allele (Choong et al., 2016). Other

approaches involve up or down regulation of either a single or a group of genes, or optogenetic/chemogenetic strategies (Kullmann et al., 2014). Additionally, the recent development of gene- editing technologies such as the CRISPR-Cas9 system enables the endogenous repair of pathogenic gene mutations (Colasante et al., 2020).

Gene therapy has been introduced into clinical practice in some neurological disorders with encouraging results (Deverman et al., 2018). Gene therapy for epilepsy also shows promise. However, to date, this remains at the stage of laboratory testing. Considerations for gene therapy in epilepsy include:-

1. the need to select the appropriate viral vector – the virus needs to be able to achieve stable transduction of neuronal populations, needs to have the capacity to package the gene of interest, and needs to have low risks of immunogenicity;
2. the need for cellular selectivity; and
3. the need to maintain normal splicing and post-transcriptional processing (Colasante et al., 2020, Steriade et al., 2020).

For Dravet's syndrome, since inhibitory GABA-ergic interneurons are the neuronal population responsible for *SCN1A* haploinsufficiency (**Section 7.3.1**), gene therapy using an adeno-associated virus (AAV) vector with regulatory sequences to target GABA-ergic cortical inter-neurons has been developed. Intra-cerebral injection of this virus vector in mouse models of Dravet syndrome has been shown to lead to reduced seizure frequency, a better behavioural phenotype and lower mortality. Human clinical trials are planned (Steriade et al., 2020).

For LKS and *GRIN2A* specifically, a few factors may need to be considered before clinical initiation of gene therapy. Firstly, there is the question of when and who do we treat. For most existing gene-therapies, the best results are achieved when therapy is administered early, preferably at the pre-symptomatic stage (Deverman et al., 2018). Although *GRIN2A* does not have high expression at birth (**Chapter 4, Section 4.1**), we do not know how effective gene therapy would be if this is carried out after the onset of symptoms. Since most *GRIN2A* mutations identified in LKS are familial, it would be possible to screen family members for mutations before they become symptomatic. However, given the considerable phenotypic variability with *GRIN2A* mutations, it may

not be possible to predict the eventual severity of a pre-symptomatic carrier's phenotype. Some individuals may turn out to be relatively asymptomatic. This raises ethical considerations on whether every carrier should be treated. It may also confound eventual assessments on treatment efficacy.

Secondly, which cell populations should we target and what dosage do we use? *GRIN2A* is widely expressed in the brain and targeting the wrong neuronal populations or using the wrong dosage may result in adverse neuro-toxic effects. As mentioned above (**Section 7.3.1)**, it has been proposed that, like Dravet Syndrome, inhibitory inter-neurons expressing *GRIN2A* may be responsible for *GRIN2A* haploinsufficiency having overall excitatory effects. To my knowledge, to date, no experimental study has established this. Should this also be the neuronal population to target for LKS? Alternatively, would hippocampal neurons be a better target considering this is the region established to be important for learning and memory and from which abnormal epileptiform discharges typically arise?

Lastly, LKS is genetically heterogeneous and *GRIN2A* accounts for only the minority of LKS individuals.

In summation, whilst gene therapy as an option for LKS may not be very far in the future, there is still much we need to uncover regarding pathogenic mechanisms for LKS, before this becomes viable.

7.4 Limitations and future directions

"*We are not beginners forever, but we never stop learning.." Sandra Scofield*

This PhD study has taught me so much, not least that, in the limited time period I have, I am not able to uncover all the answers I seek. This research will have to go on, beyond the realms of this PhD in search for more answers, for greater understanding of the complex mechanisms governing epilepsy and neuro-cognition.

7.4.1 Clinical Study

The accumulation of this large LKS cohort within a single centre over the course of nearly 30 years, affords the unique opportunity to learn so much about the clinical course of this disorder and optimal management strategies.

The limited time I have had for this PhD meant that I only had enough time to collect phenotypic data for 70 individuals (**Chapter 3**). However, we have consent to collect phenotypic data for 91 individuals. Given more time, it will be useful to complete this clinical study to give us a statistically better powered study to look at what factors affect language and long term outcome.

In addition, the study carried out by the Developmental Epilepsy clinic team at Great Ormond Street hospital correlating neuropsychological comorbidities with EEG findings and steroid therapy in 49 LKS patients has had fascinating results. It would be interesting to extend this study, within this cohort, or possibly to carry out a similar study prospectively, involving other LKS cohorts with a standard steroid therapy protocol, to see if these results can be replicated consistently.

7.4.2 Genetic analysis

The genetic analysis of this cohort cannot be deemed complete without addressing the limitations of what I have been able to carry out to date.

Firstly, in the genetic analysis of this LKS cohort, we have performed a thorough search for variants in genes that occur *de novo* within triomes, or that have occurred with recessive or X-linked recessive inheritance. We have identified a few interesting gene candidates, but these will need corroboration from other LKS cohorts and functional investigations to determine their role in the aetiology of LKS. These will be important next steps to take to bring the results of this study forward.

Secondly, within our *GRIN2A* negative LKS cohort, there are 2 families with 2 affected siblings and one family with an affected father and 3 affected children. For the former, autosomal and X-linked recessive inheritance analyses have been carried out but they will require autosomal dominant inheritance analysis; for the latter, autosomal

dominant inheritance is the only likely inheritance pattern. Analysis for autosomal dominant inherited variants for these families has commenced. However, as filtering for autosomal dominant variants in each family can yield more than a thousand potentially pathogenic variants per triome, this will take more time to complete.

Thirdly, most of the *GRIN2A* negative cohort had DNA sent for whole genome sequencing. These individuals were screened for copy number variants (CNVs) via LUMPY as described in **Chapter 2, 2.3.5** (Layer et al., 2014). 22 probands had whole exome sequencing and it was not possible for us to screen for CNVs via LUMPY. Amongst these 22 patients, 1 has had CNV screening via the diagnostic laboratory at his referring hospital. For the remaining 21 patients, we are arranging for patients with ongoing follow up at Great Ormond Street's Developmental Epilepsy Clinic to have CNV screening via diagnostic single nucleotide polymorphism microarray. It may not be possible for us to arrange screening for patients who are no longer under active follow up at any neurological clinic and that is a limitation for this study. CNVs are known to play an important role in the pathogenesis of neurological disorders, and it would be ideal to fill this gap in our current genetic analysis (Myers and Mefford, 2015).

Fourthly, even though some of this cohort have had whole genome sequencing, we have yet to look for significant variants within deeply intronic regions, as we filtered for variants that were no more than 20 bases from the exon border. To complete genetic analysis of this cohort, it would be important to interrogate the intronic regions, to look for important variants in promoter or other gene regulatory regions.

Fifth, the methods used for whole exome sequencing and whole genome sequencing in this study have not enabled us to look for microsatellite expansion, such as trinucleotide repeats (TREs). TREs have been identified as a cause of an increasing number of neurological disorders and, in future, it will be important to exclude these as an important contributory factor in the aetiology of LKS (Liu et al., 2017b).

Sixth, the methods we have used in this study have not enabled investigation of epigenetic mechanisms. In future, it may be interesting to carry out investigations such as genome- wide methylation analysis, to look at differential methylation as a cause for LKS, particularly within families with genetic variants with incomplete penetrance.

Seventh, it would be interesting to carry out weighted burden analysis to determine if there is an excess of variants in particular pathways (e.g. the LTP pathway) within the LKS cohort.

Lastly, within this cohort, families who were found to harbour *GRIN2A* mutations received no further genetic investigation. Considering the high rate of incomplete penetrance, and the possibility of LKS being multi-factorial and needing a "second-hit" before symptoms manifest, it may be important to also interrogate these families for other novel gene candidates that may confer disease susceptibility or be part of the oligogenic profile of LKS.

7.4.3 Immunological studies

Since the millennium, there has been a paucity of studies looking at the role of autoimmunity in Landau Kleffner Syndrome. It may be important to resume the search for auto-antibodies in this disorder, perhaps starting with auto-antibodies acting against important proteins along the LTP pathway.

7.5 Conclusion

We have demonstrated that LKS is a clinically heterogeneous disorder and that it is likely to have multi-factorial aetiology. It is not known if all aetiological factors converge on a common pathway, perhaps one involved in long term potentiation/ learning and memory mechanisms. If so, is there one management option that can modulate this pathway leading to the resolution of symptoms, or should each individual be treated with treatment targeted to his/her aetiology?

I am grateful to have had the opportunity to conduct this LKS study. It has given me significant insight to the interaction between seizures, and neuro-cognition/behaviour, that are likely to have wide application in the field of paediatric neurology/neuropsychiatry. Learning is a process that is life-long and I look forward to learning much more, in order to, one day, be able to make more of a difference for children with LKS, and perhaps others with similar epileptic encephalopathies.

8 Appendix: Patient case histories and genetic findings

8.1 Genetic findings in GOSH LKS Cohort

Table 8-1: Summary of genetic findings in GOSH LKS Cohort

	Total number of cases	**Cases**
GRIN2A- positive (not sent for WES/WGS)	7	Classical: 9, 11, 49, 67, 68, 79, 95
More than 1 variant on WES/WGS	27	Classical: 3, 7, 8, 41, 44, 46, 48, 53, 61, 63,66, 74, 78, 82, 89
		Atypical: 30, 43, 50, 52, 57, 64, 75, 81, 84, 86, 90
1 variant on WES/WGS	13	Classical: 24, 26, 29, 34, 45, 55, 62, 65, 70, 72, 73
		Atypical: 16, 58, 60
0 variants on WES/WGS	12	Classical: 2, 4, 20, 21, 33, 35, 40, 51, 59, 71, 80, 88
		Atypical: 93, 96

The clinical histories and gene variants of all the GOSH LKS cohort patients with positive findings have been summarised in the following sections.

8.2 *GRIN2A* positive LKS

Case 9 (Classical- GRIN2A positive)

Case Summary:

Case 9 is a girl with normal early development. She presented at 5 years of age with speech and language regression (to the level of becoming non-verbal), and challenging behavioural difficulties. She had some response to steroid therapy, but relapsed at the age of 9 years 4 months. At her last formal assessment at the age of 9 years 6 months, her language skills fell within the severe impairment range. As an adult, she is independent but continues to have significant speech and language difficulties.

Early development: Normal

Co-morbidities: Behavioural difficulties – attention deficits and aggression, mild ID

Family history: NIL

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: No lateralising features.

EEG: Frequent discharges in left parietal and left posterior temporal lobe. No sleep EEG results available

Genetic findings: *GRIN2A:* c.2041C>T, p.Arg681*

Case 11 (Classical- GRIN2A positive)

Case summary:

Case 11 presented at the age of 3.5 years with left focal motor seizures. He was started on anti-convulsants with limited efficacy. He was reported to always have had inarticulate speech, however, at the age of 8 years, he had a clear regression of his speech and language skills to dense receptive and expressive aphasia. He was also noted to have difficulty in both fine and gross motor skills, giving him difficulty with hand-writing and physical education classes. This was helped by physiotherapy and occupational therapy. He had clear improvement on steroid therapy, but relapsed when this was stopped. He received a second course of steroids with good response. At his last formal assessment at 14 years 9 months of age, he had moderate impairment in his speech and language abilities. As an adult, he has ongoing seizures. He is independent with some ongoing speech and language difficulties. He works as a porter in a hotel.

Early development: Speaking in 2 to 3 -word sentences by 2 years of age, but speech was thought to be inarticulate. Otherwise, within normal limits.

Co-morbidities: Behavioural difficulties – aggression and tantrums. Motor difficulties: gross and fine motor –dyspraxia. Mild ID.

Family history: Younger brother has seizures and speech and language difficulties.

Dysmorphic features: NIL

Examination findings: Motor dyspraxia and fine motor difficulties – difficulty threading beads; otherwise normal neurological examination

MRI: Normal

EEG: Frequent sharp and slow waves over both hemispheres, maximal in the centro-temporal regions. Sleep EEG not available.

Genetic findings: *GRIN2A* c.1552C>T**;** p.Arg518Cys

Case 49 (Classical- *GRIN2A* positive)

Case summary:

Case 49 is a girl who started having right focal motor seizures at 4 years 7 months of age. Her seizures were generally well controlled on anti-convulsants. At 6 years 10 months of age, her teachers noted some deterioration in her language particularly with language processing difficulties. She was started on speech and language therapy with some reported improvement. At the age of 9 years, she was noted to have increased seizure frequency and another episode of deterioration in her language skills. She became more withdrawn and less communicative. She was started on Clobazam with electro-clinical improvement. She became more responsive and had better speech. At her last assessment, her language skills fell within the moderate impairment range.

Early development: Speech and language delay, otherwise within normal limits

Co-morbidities: Motor dyspraxia interfering with writing

Family History: Younger brother with speech and language difficulties and dyspraxia

Dysmorphic features: NIL

Examination findings: Motor dyspraxia, otherwise normal neurological examination

EEG: Bilateral frequent discharges in parasagittal and temporal regions, recurrent runs in left temporal region. ESES in sleep

Genetic findings: *GRIN2A,* c.1289_1290delGT, p.Val430Glufs*18

Case 67 (Classical- *GRIN2A* positive)

Case summary:

Case 67 is a boy who was referred to speech and language therapy in nursery due to early speech and language delay. He was making good progress when at the age of 6 years 10 months he presented with speech regression (to the point of becoming non-verbal) and challenging behaviour. He did not have clinical seizures but his EEG showed signs of ESES. After he was commenced on sodium valproate and prednisolone, his expressive speech returned. However, after steroids were weaned off, he had a relapse with deterioration in his speech and behaviour. He was treated with another course of prednisolone and again achieved some improvement. At his last clinic assessment at 8y4m of age, he was able to speak in short sentences. He scored within the severe impairment range for his language skills.

Early development: Speech and language delay, otherwise within normal limits

Co-morbidities: Motor dyspraxia interfering with writing, mild ID, behavioural difficulties

Family History: Brother had learning difficulties and speech and language impairment

Dysmorphic features: NIL

Examination findings: Motor dyspraxia, otherwise normal neurological examination

EEG: Frequent focal sharp and slow waves in the left centrotemporal regions, with some independent focal sharps in the right centrotemporal region. ESES in sleep,

Genetic findings: *GRIN2A,* c.1776_1777dupAA, p.Ala593Lysfs*62

Case 68 (Classical- *GRIN2A* positive)

Case summary:

Case 68 is a girl with a history of early speech and language delay. She made significant progress with speech and language therapy. However, from the age of 5 years 4 months, her speech gradually deteriorated to unintelligible babble. Her behaviour became extremely challenging to manage and she was noted to have motor dyspraxia with coordination difficulties. She has not had any clinical seizures. She had a favourable response to steroid therapy but has continued to have behavioural difficulties. At her last assessment at 7 years 4 months of age, her language abilities fell within the severe impairment range.

Early development: Speech and language delay otherwise within normal limits

Co-morbidities: Motor dyspraxia; behavioural difficulties

Family History: NIL

Dysmorphic features: NIL

Examination findings: Motor dyspraxia, otherwise normal neurological examination

EEG: Frequent sharp waves in the central regions, bilaterally, with a left sided emphasis in wakefulness. Sleep activation up to 67%.

Genetic findings: *GRIN2A*, arr16p13.2p13.13 (9,287840- 10,889,600) x1

Case 79 (Classical- *GRIN2A* positive)

Case summary:

Case 79 is a girl who presented at the age of 4 years and 2 months with generalised tonic-clonic seizures from sleep. At the age of 5 years 6 months, she sustained minor head injury after a fall at school. Her parents reported that the next day she seemed aggressive and short-tempered. 2 days later, after waking from sleep, she seemed to only be able to speak gibberish that was impossible to understand. She also seemed to be unable to understand what was said to her. Along with these, her parents reported unsteady gait, and that she seemed unable to "keep still". On examination, it was noted that she was clumsy with a broad-based gait and that she had low amplitude, dyskinetic, involuntary movements. She had a clear improvement in seizure burden, movement and speech and language skills on escalation of her anti-convulsant therapy. A further improvement was made after steroid therapy. At her last clinic review at the age of 6 years and 2 months, she scored within the average range for speech and language assessment.

Early development: Normal

Co-morbidities: Below average non-verbal skills; motor difficulties

Family history: NIL

Dysmorphic features: NIL

Examination findings: Noted to have dyskinetic movements, dyspraxia and clumsy gait on first examination. Otherwise, normal examination

MRI: Normal

EEG: Frequent multifocal discharges over both hemispheres. ESES in sleep

Genetic findings: *GRIN2A* c.1553G>A; p.Arg518His

Case 95 (Classical- *GRIN2A* positive)

Case summary:

Case 95 is a boy who had some early speech and language delay before the age of 2 years. By the age of 3 years, he had acquired quite a few words and some phrases, but at age 3.5 years, he stopped talking completely. He did not have any clinical seizures and at this time, he was subject to child protection services and fostered out, so this was initially attributed to selective mutism. It was not clear how long he remained non-verbal, but approximately a year after he was reunited with his birth mother, when he was about 6 years of age, he started regaining a few single words spontaneously. When he was 8 years 9 months of age, his younger brother presented with seizures and speech regression. This prompted his referring clinician to also perform an EEG for Case 95, and this showed evidence of ESES. He was started on sodium valproate with some reported limited improvement in his speech and language skills. At his last GOSH DEC assessment, steroid therapy was discussed and his mother elected to consider this locally with his referring clinician.

Early development: Speech and language delay

Co-morbidities: NIL

Family history: Mother and brother with history of seizures and speech and language delay. Younger brother later diagnosed with LKS.

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Frequent focal sharp and slow wave activity over the right hemisphere and asynchronously over the left hemisphere in the centro-parietal and fronto-temporal regions, ESES in sleep.

Genetic findings: *GRIN2A* c.3212_3221del; p.His1071Leufs*33

8.3 *GRIN2A* -negative Classical LKS

Case 3 (Classical LKS)

Case summary:

Case 3 was born prematurely at 35 weeks of gestation, and stayed for 24 hours in the neonatal unit. He was reported to have some mild speech and language delay at 2 years of age and started having challenging behaviour when he started nursery at 2-3 years of age. He started having nocturnal right focal motor seizures at 3 years 4 months of age. Some of these were prolonged and were associated with right Todd's paresis. With the onset of his seizures there was gradual deterioration in his language and further escalation of his behaviour. By 5 years 8 months of age, although his clinical seizures were controlled on AEDs, he had lost almost all speech, and stopped responding to sound. He had some response to steroid therapy, his behaviour improved and he started saying a few words and becoming more aware of sound. However, he continued to have significant difficulty with speech and language and learnt Makaton. At 7 years of age, he underwent left temporal sub-pial transections. This was of limited benefit. As an adult, he is independent and employed as a chef. He uses sign language for communication.

Early development: Mild speech and language delay.

Co-morbidities: Severe behavioural difficulty – aggression, ADHD, impulsivity, faecal smearing, ASD traits

Family history: Mother had epilepsy in adulthood, and difficulties with psychosis and depression. Maternal uncle (mother's brother) had epilepsy in childhood. Older brother had learning difficulties. 1 son with a diagnosis of LKS, 2 other sons with ASD, 1 healthy daughter.

Dysmorphic features: NIL

Examination findings: Intermittent reduced use of right upper limb. Otherwise no focal neurological signs.

MRI: Normal

EEG: Left spike and slow wave discharges over mid and posterior temporal regions with some independent discharges on the right, becoming almost continuous in sleep with limited spread to surrounding areas and opposite hemisphere.

Genetic findings for Case 3

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
DOCK8 (AS) c.5602G>C p.V1868L	Moderate to high	Encodes Dedicator of cytokinesis 8, a guanine nucleotide exchange factor (GEF) that activates the Rho-GTPase, CDC42. This protein regulates dendritic cell migration and is known to have an important role in the immune system. Its role in the CNS is not well-established. DOCK8 has also been shown to have a role in neuro-inflammation through mediating microglial migration and phagocytosis.[1] It has also been suggested though not proven that DOCK8 may have a role in polarized axon growth through its interaction with CDC42 like other members of the DOCKC group DOCK6 and DOCK7.[2]	• NDD with ID and prominent BD (MA) - 1 patient with speech regression, and CTS on EEG with no seizures[3] • ASD[4] • ID[2]	• Gene function may have a possible link to LTP/Sz mechanisms • Significant overlap in PDP and LKS-ASD, ID, SLI, BD, CTS • Gene variants identified in 3 families in this LKS cohort. All 3 probands had prominent BD like PDP associated with this gene	• For 2 families (Cases 44 & 74), identified variants in *DOCK8* were inherited from unaffected parents, although IP is reported for NDD associated with this gene. • All variants classified as VUS
PYGL (AS) c.2029A>C; p.T677P	Low	Encodes glycogen phosphorylase, responsible for glycogen degradation.[5] Role in CNS is not clear	• Glycogen storage disease VI (BA)	• 2 cases in this LKS cohort with variants in this gene, one is *de novo* and one is shared between affected father and son • Although this gene is known to cause disease in biallelic form, predictions suggest it could also possibly be haploinsufficient- %HI: 6.92, HIPRed: 0.49	• This gene is not highly expressed in the CNS and no clear role/function has been established for this gene in the CNS • Variants in this gene have not previously been associated with LKS/EASD/primarily neurological phenotype

[1](Namekata et al., 2019); [2](Griggs et al., 2008); [3](Krgovic et al., 2018); [4](Wang et al., 2016); [5](Aeppli et al., 2020)

Case 7(Classical LKS)

Case summary:

Case 7 is a girl who had normal development until the age of 3.5 years, when there was a plateauing of her speech and language skills. She started having seizures, characterized by eyelid-fluttering, staring, then loss of consciousness from the age of 4 years 3 months. Shortly after this, her parents noted that her speech was less articulate, the content of her speech was confused and she started speaking less. She did not seem to understand when her parents spoke to her but extensive audiology tests proved normal hearing. She was started on Carbamazepine with some improvement in seizure burden and speech. However, after a measles booster, at 5 years 4 months, there was a dramatic deterioration of her speech to the point of her becoming non-verbal with virtually no receptive understanding. She also developed motor dyspraxia. She had good response to steroids. There was reduction in seizure burden and she started to speak in 3 to 4-word phrases again. There was however a plateauing of her language skills on stopping steroids. At 8 years of age, a repeat steroid course was administered with limited effect. She was worked up for epilepsy surgery at 12 years of age, but her parents decided against proceeding. Her language skills made gradual improvement. As an adult, she is independent and works as a hairdresser. She does not understand what is being said at times, and has some anxiety answering the telephone. She is generally fluent with expressive speech but has limited vocabulary and occasional word-finding difficulties.

Early development: Normal

Co-morbidities: Motor dyspraxia – awkward gait, difficulty with stairs

Family history: Mother's sister had epilepsy, father's brother reported to have cerebral palsy and was non-verbal.

Dysmorphic features: NIL

Examination findings: Gross and fine motor dyspraxia, no focal neurological signs.

MRI: Slightly reduced white matter bulk in right temporal region with slight alteration in grey-white signal intensity. No other lateralising feature.

EEG: Frequent spike-wave discharges arising from left posterior temporal region. Sleep EEG not available in early presentation. Telemetry performed at 12 years of age– no epileptiform discharges.

Genetic findings for Case 7

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ARFGEF2 (UF) c.742dupA p.T248fs*7	Moderate	Encodes ADP-ribosylation factor guanine nucleotide-exchange factor 2. Through activation of ADP-ribosylation factors 1 (ARF1), it regulates neuronal migration. ARFGEF2 has also been found to regulate Golgi polarization and dendrite morphogenesis in hippocampal neuronal cell development, through its interaction with RhoA.[1]	GMH, MiC, cardiomyopathy, Sz, MD, (BA)[2] WS, microcephaly, GMH (BA)[3] LKS – MRI result not available (MA)[4]	Function of gene links to important mechanisms for both Sz and LTP Missense variant identified in another LKS patient from another study	Inherited from unaffected father, although father's brother had a history of CP with no speech – no details available. No data available on IP This proband had a normal MRI and HC, but it is possible that MA variants may have a milder phenotype than individuals with BA variants.
NOTCH1 (UM) c.2835C>G p.D945E	Moderate	Encodes a single-pass transmembrane receptor protein with a role in cell-proliferative signalling in neurogenesis. In addition to its role in neurogenesis, in mature neurons, NOTCH signalling may also a role in NMDA receptor downstream synaptic potentiation and epileptogenesis, possibly through dendritic spinogenesis or through facilitation of synaptic vesicle neurotransmitter release[5].	Adams Oliver syndrome (AOS) (MA) – cutis aplasia, transverse limb abN, cardiac abN +/- NDD, Sz[6,7]	Gene function linked to LTP/Sz mechanisms Variants identified in 3 families within this LKS cohort	All variants identified in this cohort were inherited from unaffected parents – although IP has been described in relation to AOS for this gene These probands do not have cutis aplasia, limb defects or cardiac abN associated with AOS. However they have variants in distinct domains from AOS variants.*

[1](Hong et al., 2018); [2](Yilmaz et al., 2016); [3](Banne et al., 2013); [4](Conroy et al., 2014); [5](Sha et al., 2014); [6](Stittrich et al., 2014); [7](Southgate et al., 2015) * All variants identified in this cohort occur in the EGF-like calcium binding domains. AOS variants are mostly PTVs. Missense variants in AOS occurred in domains involved in ligand binding or in non-calcium binding EGF domains. It is possible that AOS variants may result in a more severe phenotype

Case 8 (Classical LKS)

Case summary:

A boy who had normal early development until the age of approximately 2.5 years. Since then, his speech and language skills gradually regressed, to the point of becoming non-verbal. He stopped responding to verbal instructions and had repeated hearing assessments which were normal. He had his first seizure at the age of 3 years 8 months- he had fallen from his bicycle, then while eating breakfast, he became unresponsive and dribbled. Other seizure types included nocturnal GTCS and right focal motor (facial twitching) seizures. At the time of assessment in GOSH DEC in 1995, he was 7 years old and was attending a school for the deaf with signing. He was found to have virtually no receptive language and very little expressive language. He showed some response to anti-epileptics and steroid therapy, with an improvement in seizure frequency and his vocabulary to about 15-20 words. This improvement was however not-sustained and he underwent multiple sub-pial transection surgery in 1996. After surgery, there was some reported improvement in his behaviour and language, however, this was again not sustained. He had a few more episodes of relapse requiring repeated courses of steroids. Clinical seizures stopped in his teenage years, but he continued to have active epileptiform discharges on his EEG. As an adult, he is non-verbal, and uses sign language as his main means of communication. He remains on AEDs, attends a day-centre and lives with his parents.

Early development: Normal

Co-morbidities: Behavioural difficulties- obsessive traits and aggression, fine motor difficulties, severe LD

Family history: NIL significant

Dysmorphic features: NIL

Examination findings: Normal neurological examination, weight and height: 50^{th} to 75^{th} centile, head circumference 75^{th} centile

MRI: 7 years 2 months: right choroid cyst, slight asymmetry with right temporal horn and hippocampus appearing larger with increased signal in the right anterior temporal region.

EEG: Frequent spike and sharp wave activity in the left centro-temporal regions with both synchronous and independent activity over the right centro-sylvian regions. ESES in sleep.

Genetic findings for Case 8

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
IQCA1 (*de novo*) c.357+1G>T	Low to moderate	A component of the nexin-dynein regulatory complex, involved in the maintenance of the distal axoneme and the regulation of ciliary motility. Its role in the CNS is not well established.[1]	• NIL	• De novo splice site variant that is absent from all PDB	• Gene function in the CNS is unknown • Identified in only 1 case in this cohort, not previously a/w LKS
CACNA2D1 (UF) c.2930C>T; p.S977L	Moderate	Encodes the alpha-2/delta subunit of voltage-dependent Ca channels[2]. This protein has a role in the trafficking of voltage-gated Ca channels and has a critical role in synaptogenesis including the recruitment and stabilization of NMDA receptors on the post-synaptic membrane[3]	• Epilepsy and ID (CNV, MA)[2] • CNVs in CECTS (MA)[4] • WS (MA)[5] • ASD (MA)[6]	• Gene function linked to LTP/Sz mechanisms • Some overlap in PDP with LKS – ASD, seizures, CTS, ID	• Inherited from unaffected father, however IP is reported in cardiac syndromes – thought to be due to variation in other channel genes[7] • Found in only 1 case and not previously a/w LKS
NOTCH1 (UF) c.949G>A p.G317S	Moderate	Encodes a single-pass transmembrane receptor protein with a role in cell-proliferative signalling in neurogenesis. In addition to its role in neurogenesis, NOTCH signalling may also a role in NMDA receptor downstream synaptic potentiation and epileptogenesis, possibly through dendritic spinogenesis or through facilitation of synaptic vesicle neurotransmitter release[8].	• Adams Oliver syndrome (AOS) (MA) – cutis aplasia, limb abN, cardiac abN +/- NDD, Sz[9,10]	• Gene function linked to LTP/Sz mechanisms • Variants identified in 3 families within this LKS cohort	• All variants identified in this cohort were inherited from unaffected parents – although IP has been described in relation to AOS for this gene • These cases do not have abN a/w AOS. However, these variants are in distinct domains from AOS variants*
MYH7B (UM) c.5201T>G p.L1734R	Moderate to high	Encodes myosin heavy chain 7B, an actin binding protein involved in maintaining excitatory synaptic function by regulating dendritic spine structure and AMPAR trafficking in the hippocampus.[11]	• NIL neurological	• Gene function links to LTP/Sz mechanisms • Variants in this gene were identified in 4 families in this cohort	• Some variants identified in this cohort, were inherited from unaffected parents, no data on IP available

[1](Bower et al., 2013) [2](Vergult et al., 2015); [3](Risher et al., 2018); [4](Addis et al., 2018); [5](Hino-Fukuyo et al., 2015); [6](Iossifov et al., 2014); [7](Templin et al., 2011); [8](Sha et al., 2014); [9](Stittrich et al., 2014); [10](Southgate et al., 2015), [11](Rubio et al., 2011); * All variants identified in this cohort occur in the EGF-like calcium binding domains. AOS variants are mostly PTVs. Missense variants in AOS occurred in domains involved in ligand binding or in non-calcium binding EGF domains. It is possible that AOS variants may result in a more severe phenotype

Case 24 (Classical LKS)

Case summary:

Case 24 is a boy who had normal early development. At the age of 6 years, his mother reported he was ignoring her instructions and requests. Hearing tests indicated normal levels of hearing. Over a course of 2 weeks, his speech rapidly deteriorated, becoming slow and slurred then incomprehensible. At his worst, he was speaking unintelligible gibberish and had a dense auditory agnosia, not recognizing environmental sounds such as a telephone ringing. During this time, he was very frustrated and distressed. He did not have any clinical seizures but EEG showed signs of ESES. After about 3 weeks, his speech and language spontaneously improved. However, he continued to have epileptiform discharges on EEG and he continued to have challenging behaviour with aggression, ADHD and ASD traits. He was started on sodium valproate which resulted in resolution of his epileptiform discharges and gradual improvement in his behaviour. As an adult, he is independent with no significant language difficulties.

Early development: Normal

Co-morbidities: ASD, attention difficulties, behavioural difficulties – aggression

Family history: NIL

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Left centrotemporal epileptiform discharges, ESES in sleep

Genetic findings for Case 24:

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
CSPP1 (UF) c.2736A>C p.R912S	Low	Encodes centrosome spindle pole associated protein 1, a core centrosomal protein that has a role in the regulation of ciliary axoneme formation. It is implicated in Hedgehog signalling pathways important for cell-differentiation.[1]	• Joubert Syndrome (BA)[2] • CECTS (CNV, MA)[3]	• Previously identified in CECTS – with high phenotypic overlap with LKS • 2 families in this LKS cohort have variants in this gene	• Inherited from unaffected father, although intrafamilial clinical heterogeneity is recognised in CSPP1 related ciliopathies[4] • Both variants in this cohort were classified as VUS • Both patients in this cohort had much milder symptoms than Joubert Syndrome and normal MRIs, however MA variation may be associated with a milder phenotype • Whilst previously described in a patient with CECTS – this was as part of a CNV which included another gene *ARFGEF1*, a better DEE candidate gene.

[1](Frikstad et al., 2019); [2](Shaheen et al., 2014) [3](Addis et al., 2018); [4](Ben-Omran et al., 2015)

Case 26(Classical LKS)

Case summary:

A boy with a history of mild speech and language delay (1st words at 2 years) who presented at the age of 2 years with infrequent seizures. His seizure semiology included nocturnal focal motor seizures, GTCS, and non-convulsive seizures. Between 2-5 years of age, his seizures were infrequent and he continued to make good developmental progress. However, from 5-6 years of age, he had frequent prolonged seizures. During this time, he had significant language regression to the point of becoming non-verbal and relying on gestures. His EEG showed ESES. He had dramatic recovery almost back to baseline with a course of steroids, but had recurrent relapses on withdrawal of steroid therapy. He was maintained on twice weekly pulsed oral steroids and his language skills first plateaued then showed signs of gradual recovery. Clinical seizures ceased at 11 years of age. At his last assessment at 14 years of age, he was seizure free and had functional speech with only mild impairment on formal testing. As an adult, presently, he is in independent employment with no significant speech and language difficulties.

Early development: Mild speech and language delay, otherwise normal.

Co-morbidities: NIL significant

Family history: Mother and older brother had a history of febrile convulsions.

Dysmorphic features: NIL

Examination findings: Normal neurological examination. Weight: 75th to 90th centile, height: 75th centile, head circumference: 50th centile.

MRI: Normal

EEG: Multifocal epileptiform discharges, most prominent in the left temporal region. ESES in sleep.

Genetic findings Case 26

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
CDH9 (AM) c.1112A>C p.K371T	Moderate	Encodes a type II classical cadherin from the cadherin superfamily, integral membrane proteins that mediate calcium-dependent cell-cell adhesion.[1] CDH9 regulates synapse development in the hippocampus and has an integral role in establishing neuronal connections.[2]	• ECSWS[3] • ASD[4] • Schizophrenia[5]	• Function of gene suggests possible link with LTP and Sz. • Significant overlap of clinical features for previously described phenotypes and LKS: EASD, SLI, ASD	• Inherited from mother who only has febrile seizures. Reduced penetrance/phenotypic variability is described in ASD, and in other cadherin-family genes ascribed to the fact that different combinations of cadherins can function in matching an axon to its target.[6,7] • Only 1 case in this LKS cohort • Classified as VUS

[1](Shimoyama et al., 2000); [2](Williams et al., 2011) [3](Lesca et al., 2012); [4](Redies et al., 2012); [5](Chen et al., 2017c); [6](Berg and Geschwind, 2012); [7](Rebsam and Mason, 2011)

Case 29 (Classical LKS)

Case summary:

Case 29 is a boy who was non-verbal at 18 months of age. At 2.5 years of age, with speech and language therapy, he had developed a few single words. At this time, he started having seizures characterised by eye-blinking and unresponsiveness. He commenced on treatment with sodium valproate. After this was started, he developed a much better vocabulary and started speaking in phrases. Unfortunately, at 4 years 3 months of age, after a viral illness, he had escalation of his seizures and speech and language regression to the point of not understanding any verbal instructions and becoming non-verbal. There seemed to be some response to steroid therapy but this was not sustained. He went on to relapse another 2 times and with each relapse, response to steroid therapy was unclear. As his behavioural difficulties escalated with steroid therapy, long term steroid therapy could not be maintained. At the age of 5 years, his seizure frequency escalated and he went into status epilepticus which needed to be treated with 5 days of barbiturate coma. After he recovered, he had no understanding of environmental sounds or spoken language and was completely non-verbal. He had significant behavioural difficulties. He transferred to a special school and became proficient in British Sign Language. At 9-10 years of age, his seizures gradually came under control. He started becoming more aware of language again and his speech began to return. At 11 years of age, he was able to transfer back to a mainstream school and stopped using sign language. At his last assessment at 14 years 9 months of age, he was entirely reliant on speech, scoring As in his examinations and had only mild impairment on speech and language assessment.

Early development: Speech and language delay, otherwise normal

Co-morbidities: Behavioural difficulties – aggression and tantrums

Family history: Older brother had speech and language difficulties

Dysmorphic features: NIL

Examination findings: Normal neurological examination, height, weight and head circumference on 50th centile

MRI: Normal

EEG: Bilateral synchronous and asynchronous centrotemporal discharges. ESES in sleep

Genetic findings for Case 29

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ADGRV1 (UF) c.8465C>A p.P2822Q	Moderate to high	Encodes a member of the adhesion family of G-protein coupled-receptors. Some members of this family – ADGRL1 have a role in axon guidance and synaptogenesis. ADGRV1 is found at synapses at cochlear and retinal cells and has been found to be needed for GABA-ergic interneuron development in the auditory cortex. It remains to be established if it may have a more widespread role in the rest of the CNS. [1,2]	• LGS, JME, EOAE, GE +/- ID[2]	• Gene function has possible link to LTP/Sz mechanisms • Some overlap in PDP and LKS- Sz, ID • Variants in this gene identified in 3 families within this LKS cohort	• All variants inherited from unaffected parents- however IP has been reported in relation to this gene.[2] • All variants have been classified as VUS

[1](Hamann et al., 2015) [2](Myers et al., 2018)

Case 34 (Classical LKS)

Case summary:

A boy who presented at 6 years 11 months of age with nocturnal generalized tonic- clonic seizures. His main seizure type was left focal motor seizures, at times followed by post-ictal aphasia. Other seizure types included GTCS, and other types of focal seizures with visual hallucinations. He had a history of mild speech and language delay with no need for therapy. He was making good developmental progress and was in the top 10% of his school at 7 years of age, when he gradually showed signs of language regression. His parents first noted that he kept asking if they would repeat what they said, then over the course of a year, he gradually became non-verbal. He was noted to have poor motor coordination and at his worst, he had difficulty walking. He was started on Clonazepam at 8 years of age and had good electroclinical response, but this response was not sustained. 6 months later, he was started on prednisolone and had a clear improvement in his speech back to speaking in short phrases. However, he relapsed after steroids was weaned off, and did not have as good a response on his second course. On pulsed 2 weekly steroids in combination with AEDs, he started to have a gradual recovery of his language skills. At his last assessment at 14 years of age, his language skills were within the average range for his age. As an adult, he has completed university education and is in independent employment with no significant speech and language difficulties.

Early development: Mild speech and language delay

Co-morbidities: Behavioural difficulties – aggression, motor coordination difficulties – at his worst, was not able to walk, when better, continued to have difficulty with balance and coordination e.g. could not ride a bicycle.

Family history: NIL

Dysmorphic features:

Examination findings: Inconsistent left sided facial weakness, fine action tremor, poor bimanual action imitation and bilateral brisk deep tendon reflexes noted. Otherwise within normal limits.

MRI: Normal

EEG: Bilateral centro-temporal discharges (right> left), with ESES in sleep

Genetic findings for Case 34

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
CTNNA3 (UM) c.1532-1G>C	Moderate	Encodes Catenin Alpha-3 or Alpha-T-catenin, an actin binding protein, and an important part of the catenin-cadherin cell-cell adhesion.[1] There is some evidence that this protein may have a role in stabilising dendritic spines.[2] This protein may also play important roles in maintaining cell-junctions in neuronal structures and cell-signalling within the cerebellum, functions which have been implicated in speech and language development and autism[3,4].	• ECSWS, LKS (MA, CNV)[5] • CECTS, ADHD and LD (MA, CNV)[6] • ASD (MA/BA)[7,8] • AD[9]	• Function of gene may have possible importance in speech and language/social development • CNVs previously reported in LKS, and other related EASD • Other previously reported phenotypes have some overlap with LKS – ASD, memory impairment • 3 cases within this cohort, 2 of which may result in splicing defects	• All variants found in this cohort were inherited from unaffected mothers. Incomplete penetrance with this gene has been reported in cardiac disorders.[10] • All variants classified as VUS

[1](Chiarella et al., 2018); [2](Abe et al., 2004); [3](Folmsbee et al., 2016); [4](Hampson and Blatt, 2015); [5](Lesca et al., 2012); [6](Addis et al., 2018); [7](Butler et al., 2015); [8](Bacchelli et al., 2014); [9](Miyashita et al., 2007); [10](van Hengel et al., 2013)

Case 41 (Classical LKS)

Case summary:

Case 41 is a girl who presented with her first seizure at the age of 2 years 4 months. Her seizure types included: right focal motor seizures, GTCS and absence seizures. There were no concerns about her development until the age of 3 years 8 months when her seizure frequency escalated, and her parents reported a loss of language skills. Her speech and seizure burden improved with steroid therapy. However, she relapsed after steroids was stopped. Before presentation at GOSH DEC at the age of 9 years, she had already had 2 to 3 episodes of relapse. Anticonvulsants, IVIG and azathioprine proved ineffective at controlling her seizures or improving her speech, but each time she relapsed she showed a positive response to steroid therapy. From the age of 9-11 years, she was maintained on weekly pulsed steroids, and on this her language skills plateaued, but her parents noted that her speech would deteriorate when it was approximately time for her steroid dose. At 11 years of age, she had another relapse with ESES, seizure exacerbation, language deterioration and challenging behaviour despite being on twice weekly pulsed steroids. She had a trial of azathioprine, clobazam, nitrazepam, rufinamide and ketogenic diet, but this all proved ineffective. She eventually seemed to respond to Zonisamide, with resolution of ESES and her seizures with accompanying improvement in her language and behaviour. At her last review at 16 years of age, she was seizure free and had functional language, estimated to be at a level expected of a 5 to 6 year- old child.

Early development: Normal

Co-morbidities: ASD traits – rigid behaviour, aggression, emotional difficulties

Family history: NIL

Dysmorphic features: NIL

Examination findings: At some visits noted to have right hemiparesis – not consistent. Otherwise normal examination.

MRI: Non-specific high signal in the right parietal deep white matter thought to be related to a perivascular space or gliosis. This remained stable through repeat MRIs. No other localizing or lateralising features.

EEG: Left-centrotemporal discharges. ESES in sleep.

Genetic findings for Case 41

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
COL4A2 (*de novo*) c.4828C>T p.P1610S	Low to moderate	Encodes the α2 chain of type IV collagen, a structural component of basement membranes in several organs in the body.[1]	• FE mostly with MRI abN e.g porencephaly (MA)[1]	• Previously reported phenotypes have some overlap with LKS – Focal Sz, DD • This is a *de novo* variant within the NC1 domain, that is important for the initiation α1 and α2 chain assembly to form heterotrimeric collagen protein.[1]	• This variant is found at low frequency in normal population databases and has been classified as a VUS. • This proband has only non-specific changes on MRI and her phenotype is not as severe as the majority of those described with *COL4A2* variants. However, some cases have been reported with just mild stable non-specific changes on MRI[1]. In addition, variants in the NC1 domain have been associated with milder phenotypes[1,2] • Only 1 case in this LKS cohort. • No clear link established between the function of this gene and Sz (without structural abN) or LTP.
ZNF646 (UF) c.1391C>T p.P464L	Low	Encodes zinc finger protein 646. The function of this protein is unclear as it has not been well studied. As a zinc finger protein, it is possible, it has a role in transcriptional regulation or DNA repair[3]	• LKS (CNV, MA)[4]	• CNV encompassing this gene previously identified in an individual with LKS	• Inherited from an unaffected father • Function of this gene is unclear and this gene has not previously been associated with disease • Although previously described in an individual with LKS, this was as part of a CNV, encompassing several other genes.

[1](Zagaglia et al., 2018), [2](Jeanne and Gould, 2017), [3](Cassandri et al., 2017),[4](Conroy et al., 2014);

Case 44 (Classical LKS)

Case summary:

A boy who was born at term via normal vaginal delivery but whose neonatal period was complicated by intraventricular haemorrhage and post-haemorrhagic hydrocephalus, requiring the insertion of a ventriculo-peritoneal shunt. His early motor milestones were slightly delayed – he sat at 10 months and walked at 18 months, but there were no concerns with other developmental milestones. A speech and language assessment at 2 years of age revealed speech and language skills that were appropriate for his age. After the onset of seizures at 2 years 6 months of age, his mother reported loss of language skills, particularly with receptive skills. His seizure semiology mainly comprised right focal motor seizures, some with pallor and vomiting. His seizures and language made some recovery on Levetiracetam, Clobazam and Prednisolone, but his parents reported even better recovery with hydrocortisone administered overseas. At his last review, he was seizure free. He had some functional speech, but still scored within the severe impairment range for his age.

Early development: Mild motor delay.

Co-morbidities: Attention difficulties, ASD

Family history: NIL for epilepsy, father had a history of bipolar disorder

Dysmorphic features: NIL

Examination findings: Weight and height on 2nd centile, head circumference on 0.4th centile. Brisk reflexes in bilateral lower limbs with crossed adductor reflexes, upgoing plantar reflex on the right. Otherwise, symmetrical power. Left hand dominant.

MRI: Asymmetrical bilateral PVL with volume loss more pronounced on left hemisphere, with some signal abnormalities in the left basal ganglia

EEG: Frequent epileptiform discharges in the left posterior temporal region, with independent discharges on the right. ESES in sleep

Genetic findings for Case 44

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
DOCK8 (UM) c.3197G>T p.G1066V	Moderate to high	Encodes Dedicator of cytokinesis 8, a guanine nucleotide exchange factor (GEF) that activates the Rho-GTPase, Cdc42. Regulates dendritic cell migration in the immune system. Has a role in neuro-inflammation through mediating microglial migration and phagocytosis.[1] May have a role in polarized axon growth by interaction with CDC42.[2]	• NDD with ID and prominent BD (MA) - 1 patient with speech regression, and CTS on EEG with no seizures[3] • ASD[4] • ID[2]	• Gene function may have a possible link to LTP/Sz mechanisms • Significant overlap in PDP and LKS- ASD, ID, SLI, BD, CTS • Gene variants identified in 3 families in this LKS cohort. All 3 probands had prominent BD like PDP associated with this gene	• For 2 families (Cases 44 & 74), identified variants in *DOCK8* were inherited from unaffected parents, although IP is reported for NDD associated with this gene. • All variants classified as VUS
CHD2 (UM) c.4228G>T; p.D1410Y	Moderate	An adenosine triphosphate dependent chromatin re-modeler which regulates gene transcription.[5] *CHD2* haploinsufficiency has been shown to result in abN in neuron proliferation and excitability in murine models. Phenotypically, these mice have memory deficits.[6]	• Epilepsy (MA)[5] ▪ Overall GS>FS ▪ Phenotype usually includes DD, BD and ID • Schizophrenia (MA)[7] • ASD (MA)[5]	• Gene function can be linked with seizure manifestation and LTP processes • Some overlap in PDP and LKS – including Sz, DD, and BD, a few individuals reported with developmental regression	• Inherited from unaffected mother. However, incomplete penetrance has been reported[6] • Only 1 case in this LKS cohort, not previously described in LKS • VUS
ARHGEF4 (UM) c.1549C>T p.(Gln517*)	Moderate	Encodes a Rho- guanine nucleotide exchange factor.. ARHGEF4 inhibits synaptic localization of post-synaptic density- 95 (PSD-95), a major scaffolding protein in the post-synaptic density. Through acting as a specific GEF for the GTPase CDC-42 (cell-division cycle-42), ARHGEF4 also regulates dendritic outgrowth.[8]	• CECTS (only gene within a CNV, MA)[9] • DEE with ID, SLI, ADHD (recurrent CNV hotspot in 2q21.1 with 5 genes: *GPR148, FAM123C, ARHGEF4, FAM168B, and PLEKHB2*)[10]	• Function of gene links to mechanisms important for LTP/Sz • Overlap between LKS and PDP: CTS, SLI, ADHD • Identified in 2 individuals within this LKS cohort • Both rare mutations that are PTBP–the one is a PTV, this other is within the Dbl homology domain, essential for GEF activity,	• Inherited from unaffected parents. No available data on IP. • Both variants have been classified as VUS

[1](Namekata et al., 2019); [2](Griggs et al., 2008); [3](Krgovic et al., 2018); [4](Wang et al., 2016); [5](Chen et al., 2020a); [6](Kim et al., 2018) [7](Poisson et al., 2020); [8](Oh et al., 2018), [9](Addis et al., 2018); [10](Dharmadhikari et al., 2012)

Case 45 (Classical LKS)

Case summary:

Case 45 was referred to GOSH DEC at 10 years of age. His early development was within normal limits and he was described as a sociable and popular boy at school. He started having right focal motor seizures at the age of 5 years. At the age of 5 years 10 months, he had a rapid decline of his expressive speech to his becoming non-verbal but he had relative preservation of verbal comprehension. Following this, he developed more frequent seizures including drop attacks, absence seizures, tonic seizures and GTCS. From the age of 6 years, he had lost comprehension of speech and did not recognise environmental sounds. After a course of steroids at 6 years 3 months there was a dramatic return of his speech, with him speaking in sentences again. This benefit was not sustained and he relapsed after steroid therapy was stopped. He had slower response to his second course of steroids, but again his speech returned. Following this second course, he was maintained on several anticonvulsants and twice weekly pulsed prednisolone. At his last formal assessment, his speech was clear and in sentences although he would occasionally have word-finding difficulties. His verbal comprehension was good, although his parents reported that he tended to have difficulty in noisy environments.

Early development: Normal

Co-morbidities: Behavioural difficulties, some bulbar difficulties – drooling and chewing difficulties, motor difficulties – difficulty with handwriting and with throwing and catching things.

Family history: Younger brother diagnosed with CECTS, younger sister also had 2 seizures; paternal uncle had seizures

Dysmorphic features: NIl

Examination findings: Normal neurological examination

MRI: Normal

EEG: Independent left and right centro-temporal epileptiform discharges with activation in sleep, not meeting criteria for ESES.

Genetic findings for Case 45

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
NPRL3 (UF, AS) c.767+1G>T	High	Encodes nitrogen permease regulator 3 like protein, part of the GATOR1 (Gap activity towards Rags) complex that serves as a negative regulator of mammalian target or rapamycin pathway which in turn has many functions including cell proliferation, motility, apoptosis and synaptic plasticity[1]	• FFEVF[1,2]	• Gene function links to well-recognised LTP/Sz mechanisms • Significant overlap between PDP and LKS – FE • Identified in 2 families within this LKS cohort • Rare splice variant predicted to lead to skipping of exon 7, present in both affected siblings for this family	• Both variants in this cohort were inherited from unaffected parents. However, IP is recognised for this gene[2] • Not previously identified in LKS

[1](Baldassari et al., 2016), [2](Ricos et al., 2016)

Case 46 (Classical LKS)

Case summary:

Case 46 is a male who had normal development until the age of 4.5 years, and was bilingual and talkative. At the age of 4.5 years, he stopped responding to speech and acted "deaf", but his hearing tests were normal. He gradually lost expressive speech to the point of having virtually no receptive or expressive language. He had a single nocturnal left focal motor seizure at the age of 5 years 2 months, and was started on sodium valproate. He has had no clinical seizures since. His EEG showed signs of ESES, and he had a course of steroid therapy with minimal response. He subsequently underwent multiple subpial transections in 2010 at 9 years of age, but this was also of limited help. He had preserved non-verbal skills, and learnt sign language. He was eventually educated at a language educational placement. At his last assessment at the age of 14.5 years, he continued to have no functional language.

Early development: Normal

Co-morbidities: NIL significant

Family history: NIL

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Bilateral temporal discharges (left more than right), ESES in sleep

Genetic findings for Case 46

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
TRPC1 (*de novo)* c.961-2A>C	High	Encodes transient receptor potential cation channel, sub-family C, member-1. This channel has been implicated in epileptiform burst-firing in epileptogenesis and in hippocampal neurotransmission and LTP	• NIL	• Function of gene highly suggests importance in Sz and LTP mechanisms • *De novo* protein splice -site variant in a gene highly predicted to cause skipping of an exon • Classified as likely pathogenic on ACMG guidelines	• Variants have not previously been identified in LKS/epilepsy
CTNNA3 (UM) c.2265+5G>A	Moderate to high	Encodes Catenin Alpha-3 or Alpha-T-catenin, an actin binding protein, and an important part of the catenin-cadherin cell-cell adhesion.[3] There is some preliminary evidence that this protein may have a role in stabilising dendritic spines.[4] This protein may also play important roles in maintaining cell-junctions in neuronal structures and cell-signalling within the cerebellum, functions which have been implicated in speech and language development and autism[5,6].	• ECSWS, LKS (MA, CNV)[7] • CECTS, ADHD and LD (MA, CNV)[8] • ASD (MA/BA)[9,10] • AD[11]	• Function of gene may have possible importance in speech and language/social development • CNVs previously reported in LKS, and other related EASD • Other previously reported phenotypes have some overlap with LKS – ASD, memory impairment • 3 cases within this cohort, 2 of which may result in splicing defects	• All variants found in this cohort were inherited from unaffected mothers. Incomplete penetrance with this gene has been reported in cardiac disorders.[12] • All variants classified as VUS

[1](Bröker-Lai et al., 2017); [2](Zheng, 2017) [3](Chiarella et al., 2018); [4](Abe et al., 2004); [5](Folmsbee et al., 2016); [6](Hampson and Blatt, 2015); [7](Lesca et al., 2012); [8](Addis et al., 2018); [9](Butler et al., 2015); [10](Bacchelli et al., 2014); [11](Miyashita et al., 2007); [12](van Hengel et al., 2013)

Case 48 (Classical LKS)

Case summary:

Case 48 is a boy who had some pre-existing speech and language impairment with stuttering which improved with speech and language therapy. His speech was described to be at its best at 5-6 years of age, just before the onset of seizures at 7 years of age. His seizure semiology included: right sided focal motor seizures (some with Todd's paresis), absence seizures and atonic seizures. A few months after the onset of seizures, his parents reported slurring of speech and loss of receptive understanding. His expressive speech became slow and difficult to understand. He responded well to his first course of steroids, with improvement in seizure burden and clearer speech. However, on withdrawal of steroid therapy he had a relapse and did not respond to further repeated courses of steroids or AEDs. His seizures recurred and he became densely aphasic with severe behavioural problems. Multiple subpial transections surgery was considered, however, he had spontaneous improvement at 11 years of age, with cessation of seizures, followed by gradual steady improvement in his language skills. At his last assessment at 15 years of age, he scored within the average range for his speech and language assessment, although he reported some social anxiety over speaking in public.

Early development: Pre-existing speech and language impairment, otherwise normal.

Co-morbidities: Behavioural difficulties- impulsivity, mood-swings, ADHD.

Family history: NIL significant

Dysmorphic features: NIL

Examination findings: Found to have right sided weakness on one occasion- not found on other examinations. Otherwise normal.

MRI: Normal

EEG: Very frequent left centrotemporal spikes with some independent right sided discharges, ESES in sleep

Genetic findings for Case 48

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
CTXN3 (*de novo)* c.164C>T; p.P55L	Moderate to High	Encodes cortexin-3. The function of this gene has not been well studied, but some evidence suggests that CTXN3 may be involved in the development of GABA-ergic neurotransmission during CNS development. CTXN3 has also been implicated in the metabolism of amyloid precursor protein which influences the expression of the N1 subunit of the NMDA receptor and has roles in synaptogenesis.[1]	• Schizophrenia[1] • Differential expression of this gene has been identified in refractory epilepsy[2]	• Function of this gene may be linked to important mechanisms for LTP/Sz • De novo variant that lies within the cortexin domain of this protein, is predicted to be pathogenic by most in silico algorithms, is absent in all population databases and is classified as likely pathogenic by ACMG criteria	• Variants in this gene has not previously been reported in LKS/epilepsy • Only 1 case in this LKS cohort
CTNNA3 (UM) c.130C>T; p.P44S	Moderate	Encodes Catenin Alpha-3 or Alpha-T-catenin, an actin binding protein, and an important part of the catenin-cadherin cell-cell adhesion.[3] There is some preliminary evidence that this protein may have a role in stabilising dendritic spines.[4] This protein may also play important roles in maintaining cell-junctions in neuronal structures and cell-signalling within the cerebellum, functions which have been implicated in speech and language development and autism[5,6].	• ECSWS, LKS (MA, CNV)[7] • CECTS, ADHD and LD (MA, CNV)[8] • ASD (MA/BA)[9,10] • AD[11]	• Function of gene may have possible importance in speech and language/social development • CNVs previously reported in LKS, and other related EASD • Other previously reported phenotypes have some overlap with LKS – ASD, memory impairment • 3 cases within this cohort, 2 of which may result in splicing defects	• All variants found in this cohort were inherited from unaffected mothers. Incomplete penetrance with this gene has been reported in cardiac disorders.[12] • All variants classified as VUS

[1](Šerý et al., 2015); [2](Liu et al., 2016) [3](Chiarella et al., 2018); [4](Abe et al., 2004); [5](Folmsbee et al., 2016); [6](Hampson and Blatt, 2015); [7](Lesca et al., 2012); [8](Addis et al., 2018); [9](Butler et al., 2015); [10](Bacchelli et al., 2014); [11](Miyashita et al., 2007); [12](van Hengel et al., 2013)

.

Case 53 (Classical LKS)

Case summary:

Case 53 is a girl with a history of minor articulation difficulty when she was younger but had otherwise normal early development. At the age of 5.5 years after recovering from chicken-pox, her teachers expressed concern over her hearing as she seemed to ignore their instructions. Hearing assessments returned normal results. Over the next 3-4 months her speech then deteriorated to the point of her almost losing all expressive speech. Her behaviour became extremely challenging as she became quite aggressive, hitting and biting other children at school. She did not have any clinical seizures, but EEG showed ESES. At her first assessment, her language skills were felt to be at the 10 to 12- month age equivalent level with her expressive language just slightly better than her receptive skills. Sodium valproate was of limited benefit, and she was given a 6-week course of steroids. After steroid therapy, she had remarkable improvement in her language skills and returned to almost age- appropriate level. Unfortunately, she had a relapse after steroids were withdrawn and a second course had limited effect. This time she had a dense auditory agnosia with no comprehension of the spoken word or environmental sounds and became virtually non-verbal. She underwent multiple subpial transection at 7 years of age but this seemed to be of limited benefit, and she had ongoing speech and language impairment and challenging behaviour. Clobazam and Ethosuximide were tried but with limited benefit. Approximately 18 months after surgery however, her language and behaviour gradually improved. A repeat EEG showed no epileptiform discharges. At her last formal review at 11 years of age, her language skills fell within the mild impairment range.

Early development: Normal

Co-morbidities: Behavioural difficulties, aggression and impulsivity, ASD traits, attention difficulties

Family history: NIL significant

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Epileptiform discharges more prominent in the right fronto-centro-parietal and temporal regions, ESES in sleep

Genetic findings for Case 53

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ERRFI1 (*de novo)*	Moderate to high	Encodes a cytoplasmic protein that is up-regulated during cell-growth. There is some evidence that this is one of the genes that is induced by the MAPK/ERK signalling pathway during LTP, and that this protein has a role in the regulation of neurite outgrowth and cortical neuron migration.[1,2]	• NIL neurological	• Function of this gene can possibly link to mechanisms for LTP and Sz • This is a d*e novo* frameshift variant that is absent from all population databases and is classified as likely pathogenic by ACMG criteria	• Variants in this gene has not previously been reported in LKS/epilepsy • Only 1 case in this LKS cohort
NOTCH1 (UM) c.2182G>A p.G728R	Moderate	Encodes a single-pass transmembrane receptor protein with a role in cell-proliferative signalling in neurogenesis. In addition to its role in neurogenesis, in mature neurons, NOTCH signalling may also a role in NMDA receptor downstream synaptic potentiation and epileptogenesis, possibly through dendritic spinogenesis or through facilitation of synaptic vesicle neurotransmitter release[3].	• Adams Oliver syndrome (AOS) (MA) – cutis aplasia, transverse limb abN, cardiac abN +/- NDD, Sz[4,5]	Gene function linked to LTP/Sz mechanisms • Variants identified in 3 families within this LKS cohort	• All variants identified in this cohort were inherited from unaffected parents – although IP has been described in relation to AOS for this gene • These probands do not have cutis aplasia, limb defects or cardiac abN a/w AOS. However they have variants in distinct domains from AOS variants.*

[1](Blüthgen et al., 2017); [2](Pante et al., 2005); [3](Sha et al., 2014); [4](Stittrich et al., 2014); [5](Southgate et al., 2015) * All variants identified in this cohort occur in the EGF-like calcium binding domains. AOS variants are mostly PTVs. Missense variants in AOS occurred in domains involved in ligand binding or in non-calcium binding EGF domains. It is possible that AOS variants may result in a more severe phenotype

Case 55 (Classical LKS)

Case summary:

Case 55 is a girl who was reported to have normal early development. She spoke her first words by 1 year of age and by 18 months was naming body parts. After this age, she stopped learning new words, then gradually lost the words she knew to the point of becoming non-verbal. In pre-school, her teachers thought she had hearing problems. She developed seizures at 5 years 9 months of age- characterized by behavioural arrest then fluttering of eyelids and loss of tone. Her family also reported some challenging behaviour. An EEG performed at this time showed ESES. Sodium valproate was started but there was limited benefit. On referral to GOSH DEC at 6 years of age, she had unintelligible babble and had difficulty in discriminating environmental sounds. She was started on a trial of steroid therapy, but due to a complicated social history, did not receive this. Her sodium valproate dose was optimized. This controlled her seizures but did not improve her language skills. She had a second trial of steroids a year later when her EEG continued to show ESES. This significantly improved her behaviour but had limited effect on her language. A repeat EEG showed resolution of epileptiform discharges. At her last assessment, she was sociable and well-behaved with preserved non-verbal skills. She was able to communicate well using British Sign language but had no expressive speech or verbal comprehension.

Early development: Normal

Co-morbidities: Mild behavioural difficulties – tantrums and aggression

Family history: Father's brother had a history of speech and language impairment (no parental DNA samples available – currently raised by grandmother)

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: spike wave discharges seen independently over both left and right centro-posterior temporal regions, and, at times, frontal regions with slight right sided predominance. ESES in sleep.

Genetic findings for Case 55

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
RRN3 (NPS) c.1429G>A p.G477R	Moderate	Encodes RNA polymerase 1 Transcription factor 1A. This transcription factor activates RNA polymerase 1 which transcribes nucleolar rRNA genes important for proliferative growth including that of neurite- extending neurons. A recent study implicates this transcription factor in neural plasticity and epileptogenesis.[1]	• Different types of epilepsy including CECTS (recurrent hotspot CNV in 16p13.11, MA)[2,3]	• Function of gene linked to epileptogenesis and LTP • CNVs described in epilepsy and CECTS	• First time a missense variant in this gene has been associated with LKS/epilepsy • Only case in this LKS cohort • Classified as a VUS

[1](Vashishta et al., 2018); [2](Heinzen et al., 2010); [3](Addis et al., 2018)

Case 61 (Classical LKS)

Case summary:

Case 61 is a girl who presented to GOSH DEC at 5 years 11 months of age. She had normal early development and was reported to be advanced in all her skills. At 4 years 7 months of age, her parents noted that she did not appear to notice people talking to her, unless she was looking at them. They attributed this to a possible ear infection, although none was found on examination. This resolved spontaneously. However, at 4 years 11 months she again appeared deaf, talking loudly and turning up the volume on the television. This time however, ENT examination diagnosed an ear infection and suggestions of glue ear. From then onwards, her parents reported she would often have difficulty hearing. By 5.5 years of age, it became apparent that she did not understand speech, and her teachers became concerned that she was increasingly "in her own world". Her expressive speech also deteriorated, she repeated syllables, mispronounced words, had word finding difficulties, spoke some gibberish and had a higher pitched voice. She had preserved non-verbal intelligence and often used gesture and pictures to communicate. She did not have clinical seizures but EEG showed signs of ESES. She responded well to a course of steroids, with recovery of her language skills. She has made steady progress since. At her last assessment at 9 years 3 months of age, she scored in the superior range for both verbal and non-verbal skills.

Early development: Normal

Co-morbidities: Some behavioural difficulties – tantrums and aggression

Family history: Maternal cousin who had epilepsy as a teenager

Dysmorphic features: NIL

Examination findings: Weight and height on 9th to 25th centile, head circumference: 25th centile. Normal neurological examination.

MRI: Normal

EEG: Very frequent epileptiform spike-wave complexes over bilateral posterior temporal regions (L>R), ESES in sleep (90% activation).

Genetic findings for Case 61

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ABCA7 (BP) c.2639G>A; p.R880Q, and c.2966G>A; p.R989H	Moderate	Encodes ATP (adenosine triphosphate)-binding cassette sub-family A member 7. This protein may have an indirect role to play in long term potentiation (LTP), memory and learning as it is involved in the regulation of amyloid precursor protein (APP) processing and in the phagocytosis of amyloid-beta aggregates (ABA). Both APP and ABA are involved in many processes for LTP, including neurite outgrowth, synapse formation and neurotransmitter release.[1,2]	• Risk for AD[1]	• Gene function may link to LTP/Sz processes • Both rare variants located within the ABC-transporter like and ATP-ase core domain important for ATP binding and hydrolysis. Both variants are PTBP by most in-silico algorithms	• Variants in this gene have not previously been associated with LKS/EASD • Only identified in 1 patient within this LKS cohort
PYGL (*de novo)* c.1727G>A; p.R576Q	Low	Encodes glycogen phosphorylase, responsible for glycogen degradation.[3] Role in CNS is not clear	• Glycogen storage disease VI (BA)	• *De novo* mutation that is PTBP by many in silico algorithms • 2 cases in this LKS cohort with variants in this gene • Although this gene is known to cause disease in biallelic form, predictions suggest it could also possibly be haploinsufficient- %HI: 6.92, HIPRed: 0.49	• This gene is not highly expressed in the CNS and no clear role/function has been established for this gene in the CNS • Variants in this gene have not previously been associated with LKS/EASD/primarily neurological phenotype • Only identified in 1 patient within this LKS cohort

[1](Aikawa et al., 2018), [2](Surguchev and Surguchov, 2020) [3](Aeppli et al., 2020)

Case 62 (Classical LKS)

Case summary:

Case 62 is a girl who presented at 3 years 9 months of age. She had normal early development. She said her first words before her 1st birthday and was putting words together before 2 years of age. Her parents recall her saying "I love you" before 2 years of age. After she turned 2 years of age, she gradually stopped speaking. She attended pre-school at 2.5 years of age, and her teachers reported that although she enjoyed playing with other children, she was non-verbal. At 3 years of age, she started babbling again, and by 3 years 9 months of age had about 10 words. However, shortly after this time she regressed again, and became non-verbal. An EEG at this point, showed ESES. She did not have clinical seizures. She had a trial of ethosuximide and a prolonged trial of steroid therapy but showed limited response. At her last assessment at 8 years 10 months of age, her cognitive abilities were within normal limits but she continued to have severe impairment of both receptive and expressive language skills and relied on British Sign Language for communication. Her EEG still showed signs of ESES.

Early development: Normal

Co-morbidities: Behavioural difficulties, mild tantrums, hyperactivity and aggression

Family history: Father's younger brother had language difficulties as a child

Dysmorphic features: NIL

Examination findings: Normal neurological examination, HC: 25th to 50th centile, weight and height: 75th centile

MRI: Normal

EEG: Frequent spike and wave complexes emerging from both temporal regions, occurring either independently or synchronously. ESES in sleep.

Genetic findings for Case 62

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- for causative/contributary significance for LKS/proband	
				For:	Against:
MYH7B (APS) c.716T>C p.V239A	Moderate to High	Encodes myosin heavy chain 7B, an actin binding protein that has been shown to have a role in maintaining excitatory synaptic function by regulating dendritic spine structure and AMPAR trafficking in the hippocampus.[1]	• NIL neurological	• Gene function links to important mechanisms in LTP/Sz • Variants in this gene were identified in 4 families within this LKS cohort	• Some variants identified in this cohort, were inherited from unaffected parents, no data on IP available

[1](Rubio et al., 2011)

Case 63 (Classical LKS)

Case summary:

Case 63 presented to GOSH DEC at the age of 5 years. She had her first GTCS at 3 months of age. She was admitted to her local hospital where infection and neurometabolic investigations returned normal results apart from transient derangement of liver function enzymes. She continued to have infrequent seizures. From the age of 3 years, her seizures had more of a focal semiology characterised by right sided twitching of her mouth, drooling and post-ictal aphasia, some of these progressed to GTCS. Her early development was felt to be within normal limits. She said her first word by her first birthday and by 2 years of age was joining words together. When she was approaching her 4th birthday however, her parents noted that her speech was unclear. She had an EEG at this time which showed ESES. She had a trial of steroids after which there was a clear improvement in her speech clarity. However, this was not sustained and a few months after steroid therapy was discontinued, her speech deteriorated. She had 2 further courses of steroid therapy, both of which resulted in clear improvement in her speech. After her 3rd course of steroid therapy, she was maintained on twice weekly low dose prednisolone and she had a reduction in her seizure burden and ongoing improvement in her speech and language skills. At her last review at 12 years 9 months of age, she was seizure free and had successfully transferred to a mainstream school with assistance from a special needs school. She was talkative and spoke in sentences. She scored in the mild difficulty range for expressive language but in the severe difficulty range for receptive language skills.

Early development: Normal

Co-morbidities: Social communication difficulties, mild ID, some difficulties with fine motor skills

Family history: Mother had a history of minor speech difficulties as a child, but did not have epilepsy. NIL else significant.

Dysmorphic features: NIL

Examination findings: Weight and height on 50th centile. Normal neurological examination

MRI: Normal

EEG: Frequent right centrotemporal discharges (some EEGs show left), ESES in sleep.

Genetic findings for Case 63

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- for causative/contributary significance for LKS/proband	
				For:	Against:
SCN1A (*de novo*) c.748G>A p.V250I	High Top candidate gene for this proband	Encodes the alpha subunit of a voltage-gated sodium channel found within the brain, that is crucial for regulating sodium influx for the creation of action potentials.[1]	• GEFS+, DS (MA)[1] • FE and DEE including WS, EIMFS[1] • EASD[2,3]	• Gene function linked to Sz mechanisms • Overlap in PDP and LKS: Sz, DD, EASD • De novo rare variant that has been classified as pathogenic	• Only case identified in this LKS cohort and variants in this gene not previously identified in LKS
BIRC6 (APS) c.8350A>G p.T2784A	Moderate	Encodes baculoviral IAP (inhibitor of apoptosis) repeat-containing protein 6, a ubiquitously expressed protein, with a role in the control of apoptosis. In the brain, BIRC6 has been found to be neuro-protective. Downregulation of this protein resulted in reduced neuronal viability and increased susceptibility of neurons to excitotoxicity.[4]	• ASD (MA)[5] • IS/LGS (MA CNV)[6] • EASD (MA, CNV)[7]	• Gene function links to mechanisms involved in neurotoxicity after Sz, and may link to epileptogenesis/ cognitive impairment • Overlap in PDP and LKS: Sz, ASD, SLI, developmental regression, EASD • Possibly de novo (APS)	• Only case identified in this LKS cohort and variants in this gene not previously identified in LKS

[1](Lossin, 2009); [2](Kivity et al., 2017); [3](Carvill et al., 2013a) [4](Sokka et al., 2005); [5](Wu et al., 2020); [6](Epilepsy Phenome/Genome Project & Epi4K Consortium, 2015); [7](Reinthaler et al., 2014)

Case 65 (Classical LKS)

Case summary:

A boy who developed focal motor nocturnal seizures at 4 years 8 months of age. Since the onset of seizures, his parents noted that his speech became unclear and that his speech and language skills fluctuated. He had difficulty understanding instructions and often asked his parents to repeat what they were saying. The first course of steroids was administered before attendance at GOSH DEC and was reported to result in minimal improvement in his symptoms so this was weaned off. At GOSH DEC, he had a second course of steroids as he continued to have ongoing ESES and significant behavioural difficulties. This time, his parents reported clearer speech and better behaviour. At his last assessment at 9 years 1 month of age, he scored within the mild impairment range for his language assessments.

Early development: Normal

Co-morbidities: Behavioural difficulties, ASD, mild ID

Family history: NIL significant. Maternal grandmother had acquired seizures after head injury

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Epileptiform discharges in left central region that become continuous in sleep, ESES.

Genetic findings for Case 65

Gene (Inheritance)/ Variant:	Significance to LKS/Proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	**Against:**
SMC1A (UM/X-linked) c.1543G>C p.V515L	Moderate to high	Encodes structural maintenance of chromosomes 1A protein, a subunit of the cohesin-core complex. This complex tethers sister chromatids together to facilitate smooth segregation during cell division. This protein has a role in gene transcription regulation and DNA damage repair.[1]	*Missense mutations and in-frame coding deletions:* • CdLS (XLD)[2] with ASD[3] • DEE with refractory sz and ID, mild CdLS facies)[2] • DEE with cluster sz, ID, DD, no CdLS features[4] *LoF* mutations: • Rett-like DEE[5] • EIMFS[6]	• Significant overlap between described phenotype of DEE with DD, and ID, with LKS • Maternally inherited SNVs in males on DECIPHER* with similar phenotypes – Sz, SLI, ID, ASD	• No features of CdLS • Only 2 isolated cases of missense variants associated with DEE[2, 4], other cases of DEE associated with LoF variants • Only 1 case in this LKS cohort • VUS

[1](Musio, 2020); [2](Huisman et al., 2017); [3](Mulder et al., 2019); [4](Oguni et al., 2019); [5](Symonds et al., 2017); [6](Gorman et al., 2017)

Case 66 (Classical LKS)

Case summary:

A girl with normal early development who presented at 8 years 8 months of age with a single GTCS. She then did not have any further seizures until 10 years 7 months of age. From this age, she had relatively frequent GTCS and was started on sodium valproate with limited response. When she was 11 years of age, her school raised concerns that she was having difficulty following instructions and speaking. Whilst she did not lose speech completely, she started speaking in shorter phrases, had word-finding difficulties and was unable to repeat sentences. Her EEG showed signs of ESES. She was started on steroid therapy with significant improvement. At her last review at 13 years of age, she had only mild impairment on language assessment.

Early development: Normal

Co-morbidities: Mild fine motor difficulties

Family history: NIL

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: Short bursts of generalized activity. At times, more focal with prominence in right posterior temporal region. ESES in sleep. Other EEGs: epileptiform discharges in right posterior temporal region.

Genetic findings for Case 66

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
HSPG2 (UM) c.3114delC p.I1039fs*2 Awaiting Sanger confirmation	Low to moderate	Encodes heparan sulphate proteoglycan 2, aka perlecan, a multi-functional protein with a role in the maintenance of extracellular matrices and various signalling pathways. It is believed to be involved in brain development, as perlecan-null mice have exencephaly but the mechanisms for this are not well understood.[1] Additionally, perlecan may have a role in maintenance of BBB integrity.[2]	• Schwartz-Jampel syndrome (BA)[1] • Dyssegmental dysplasia (BA)[1] • LKS (MA)[3]	• Variants previously identified in 5 individuals with LKS[3]	• This gene is most highly expressed in the MSK system and is expressed only at low levels in the brain. • Inherited from unaffected mother and classified as VUS, no clear data on IP
TCTEX1D2 (UM) c.329G>A p.R110H Awaiting Sanger confirmation	Low	Encodes a dynein light chain, a protein required for intra-flagellar transport important for ciliary growth and signalling.[4]	• Jeune syndrome (BA)[4] • EASD (MA, CNV)[5]	• Recurrent hot-spot CNV for EASD includes this gene[5]	• This gene's role in the CNS has not been clearly established • Although previously described in an individual with EASD this was as part of a CNV, encompassing several other genes. • Inherited from UM – although varying penetrance is reported for Jeune syndrome[4] • Only case in this LKS cohort • VUS

[73](Martinez et al., 2018); [74](Nakamura et al., 2019); [3](Conroy et al., 2014); [4](Schmidts et al., 2015); [5](Reinthaler et al., 2014)

Case 70 (Classical LKS)

Case summary:

A boy who had normal development until the age of 6.5 years. He did well at reception and his parents were told he was ahead of his peers. At 6.5 years of age, after an episode of a cold and otitis media, his parents noted that he often did not respond to them addressing him and would often say "pardon". He started to dislike school and his teachers reported that he was lip-reading. He had several hearing tests all of which were normal. As he got worse, he seemed unable to localize sound and whilst he retained some understanding, he was not able to retain long instructions. He had significant word finding difficulties and would often substitute words e.g. he would use "juice fork" instead of "spoon". He developed nocturnal GTCS at 7 years of age. He was started on sodium valproate with limited benefit. On first presentation at GOSH DEC, he had moderate impairment for both receptive and expressive language skills with worse receptive skills than expressive skills. He had a trial of steroid therapy with clear improvement in both seizure burden and language skills. He was maintained on pulsed twice- weekly steroid therapy for a period of 1.5 years. During this time, he was seizure free and maintained his language skills. He remained in mainstream school and made good progress, there was some deterioration when his prednisolone dose was reduced, so this had to be maintained. At his last assessment at 8 years 10 months of age, he had just mild impairment (16^{th} centile) for receptive language and scored within average range for expressive language (75^{th} centile).

Early development: Normal

Co-morbidities: NIL significant – no behavioural or motor difficulties documented, average non-verbal skills

Family history: Brother has dyslexia, nil else significant

Dysmorphic features: NIL

Examination findings: Normal neurological examination. Weight and height: 50^{th} centile.

MRI: Normal

EEG: Left mid-temporal epileptiform discharges with sleep activation occupying 25% to 80% of sleep recording at different times.

Genetic findings for Case 70

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
PDE4D (UM) c.1286A>G p.Q429R	Moderate	Encodes phosphodiesterase enzyme 4D, which hydrolyses cyclic adenosine-monophosphate (cAMP), a key intracellular signalling molecule. PDE4D has a role in regulating cAMP/PKA/CREB signalling in the brain and its importance in neurogenesis, and neuroplasticity is well established. A lot of research is ongoing for pharmacological regulation of PDE4D for neurocognitive disorders. [1,2]	• Acrodysotosis type 2 (AD)– severe ID, brachydactyly, nasal hypoplasia[1] • Schizophrenia/OCD (MA)[3,4] • CECTS (CNV with 4 other genes, MA)[5]	• Function of gene vital for LTP • Previous phenotypes have overlap with LKS – ID, BD, CTS	• Inherited from UM- although 1 study suggests PDE4D mediated pathways may differ between males and females.[6] • This proband does not have ID, BD or skeletal defects

[1](Gurney, 2019); [2](Wang et al., 2018); [3](Sinha et al., 2019); [4](Huang et al., 2019), [5](Addis et al., 2018); [6](Zamarbide et al., 2019)

Case 72 (Classical LKS)

Case summary:

Case 72 presented to GOSH DEC at 5 years 8 months of age. She was reported to be advanced with all her developmental milestones. Her first seizure occurred at the age of 4 years when she was ill with an intercurrent illness. She was found in bed covered in vomit, then had a prolonged seizure with nystagmus, she was transferred to Paediatric Intensive Care and was treated for an infectious encephalopathy, however all infectious and neurometabolic investigations returned negative results. She continued having frequent seizures including nocturnal focal motor seizures, GTCS, myoclonic and tonic seizures. From 5 years of age, it was apparent that she had regression of her speech and language skills. She often had word finding difficulties and her speech became slurred. She would watch television with a different language and not seem to notice. She started having tantrums and aggressive behaviour. Her seizures were refractory to treatment with anti-epileptic medication. A trial of steroids at 5 years of age improved her behaviour and speech, but had limited effect on her seizures. The benefit for her behaviour and speech was not sustained, and a second trial of steroids at 5 years 8 months of age had limited effect. At her last assessment at 7 years 9 months of age, she continued to have fluctuating abilities, and scored within the moderate difficulty range for both receptive and expressive language skills. She remained on several anti-convulsants with variable seizure control.

Early development: Normal

Co-morbidities: Behavioural difficulties

Family history: NIL

Dysmorphic features: NIL

Examination findings:

MRI: Normal, no focal structural abnormality

EEG: Slightly slow background when awake. In sleep, epileptiform discharges occurred independently over right & left fronto-central, temporal regions – with variable hemispheric dominance across serial EEGs.

Genetic findings for Case 72

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
NPRL3 (UF) c.965C>T p.P322L	High	Encodes nitrogen permease regulator 3 like protein, part of the GATOR1 (Gap activity towards Rags) complex that serves as a negative regulator of mammalian target or rapamycin pathway which in turn has many functions including cell proliferation, motility, apoptosis and synaptic plasticity[1]	• FFEVF[1,2]	• Gene function links to well-recognised LTP/Sz mechanisms • Significant overlap between PDP and LKS – FE • Identified in 2 families within this LKS cohort	• Both variants in this cohort were inherited from unaffected parents. However, IP is recognised for this gene[2] • Not previously identified in LKS

[1](Baldassari et al., 2016), [2](Ricos et al., 2016)

Case 73 (Classical LKS)

Case summary:

A boy who presented to GOSH DEC at 4 years of age with concerns regarding seizures and speech and language- led regression. He had normal development until the age of 2.5 years, when he was speaking in 3-4 word phrases with good vocabulary. At this time, his mother reported that 2-3 times a week, he would wake from sleep and have a few episodes of vomiting before falling asleep again. Shortly after the onset of this, she noted a distinct regression in his language skills, which started with stuttering and word-finding difficulties, then progressed to his speaking less and using gestures. He developed other seizure types including absence seizures and GTCS. On presentation at GOSH, his parents declined a trial of steroid therapy. On sodium valproate, there was reduction in his seizure burden and some concomitant improvement in his language skills. At his last assessment at 5 years of age, he was speaking in 3-4 word phrases and had ongoing clinical seizures.

Early development: Normal

Co-morbidities: ID, hyperactivity and concentration difficulties, autistic traits

Family history: ADHD and ASD on the maternal side of the family

Dysmorphic features: Single palmar crease on right hand, nil else specific

Examination findings: Normal neurological examination

MRI: Normal

EEG: Runs of epileptiform discharges over the right sylvian region, with generalised bursts in sleep, not meeting criteria for ESES.

Genetic findings for Case 73

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
DIAPH3 (UM) c.388A>G p.M130V	Moderate	Encodes diaphanous homolog 3, a protein that functions in the assembly of actin filaments.[1] Studies have shown that DIAPH3 has important roles in axonal guidance, neuronal migration and neurite formation in the developing brain. Inhibition of DIAPH3 pathways in murine hippocampal neurons result in reduced synaptic activity.[1]	• ECSWS (CNV, MA)[2] • ASD (BA)[1] • Auditory neuropathy (MA)[3]	• Function of gene links to important mechanisms for LTP • Previously described in ECSWS and ASD, phenotypes with significant overlap with LKS	• Inherited from unaffected mother. However, there is a FH of ADHD, ASD on the maternal side. There is no data available on incomplete penetrance. • Only 1 case in this LKS cohort • Classified as VUS

[1](Vorstman et al., 2011); [2](Lesca et al., 2012); [3](Surel et al., 2016);

Case 74 (Classical LKS)

Case summary:

A boy who presented to GOSH DEC clinic at the age of 7 years. He was noted to always be an extremely active child but did not have any developmental concerns until the onset of seizures at 4 years 11 months of age. His seizure semiology included focal motor seizures, generalized tonic clonic seizures, myoclonic absences, facial twitching, and drop attacks. His seizures were refractory to treatment with multiple anti-epileptic drugs. From the age of 5 years, there was a clear regression of predominantly speech and language skills with associated deterioration in behaviour. His speech and language skills and seizure frequency responded to a course of steroid therapy, but this significantly worsened his behaviour and had to be discontinued. At his last review, he continued to have intermittent seizures, behavioural concerns and severe speech and language impairment.

Early development: Normal

Co-morbidities: Attention deficit hyperactivity disorder, mild ID, oppositional and aggressive behaviour

Family history: Father reported to be hyperactive as a child. Distant paternal relative with childhood epilepsy.

Dysmorphic features: NIL

Examination findings: Normal neurological examination. Weight: 50th centile

MRI: Normal at 5 years

EEG: Frequent runs of centrotemporal discharges or diffuse generalized runs of spikes/sharp wave discharges with emphasis over centro-sylvian and temporal regions. ESES in sleep.

Genetic Findings for Case 74

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
DOCK8 (UM) c.5775C>G p.F1925L	Moderate to high	Encodes a guanine nucleotide exchange factor (GEF) that activates the Rho-GTPase, Cdc42. This protein has an important role in the immune system. Within the CNS, DOCK8 has been shown to have a role in neuro-inflammation through mediating microglial migration and phagocytosis.[1] It has also been suggested though not proven that DOCK8 may mediate polarized axon growth through its interaction with CDC42 like other members of the DOCKC group DOCK6 and DOCK7.[2]	• NDD with ID and BD (MA) - 1 patient with speech regression, and CTS on EEG [3] • ASD[4] • MR[2]	• Gene function may have a possible link to LTP/Sz mechanisms • Significant overlap in PDP and LKS-ASD, ID, SLI, BD, CTS • Gene variants identified in 3 families in this LKS cohort	• For 2 families (Cases 44 & 74), identified variants in *DOCK8* were inherited from unaffected parents, although IP is reported for NDD associated with this gene. • All variants classified as VUS
KCNQ3 (UF) c.1226C>G; p.P409R	Moderate	Encodes potassium voltage-gated channel subfamily Q member -3. This family of voltage gated potassium channels generates the neuronal M-current that has an important role in regulating neuronal excitability[5].	• BFNS, BFIS (MA)[5], • Non-specific DEE[6] (MA/BA)[7,8] • LGS (MA)[9] • ASD, DD (MA)[10]	• Gene function can be linked with seizures • Described ASD/DD phenotype[9] has significant overlap with LKS- mild seizures/no seizures, ESES, DD, ASD	• Inherited from his father, who did not have LKS. However, this father had a history of hyperactivity and FH of epilepsy. There are reports of IP.[11,12] • PDP generally associated with younger age of onset of seizures and less refractory seizures • Only 1 case in this LKS cohort • VUS
LTBP1 (UF) c.2222A>G p.H741R	Moderate	Encodes latent transforming growth factor β binding protein-1, which regulates the function of latent TGF- β.[13,14] Within the CNS, a study looking at the transcriptomic profiles of a murine model of TLE has identified *LTBP1* as a key regulator in epileptogenesis. [10]	• EASD (CNV, MA)[15]	• Gene function may have link to epileptogenesis • EASD[15] is PDP • Variants in 2 families in this LKS cohort	• CNV identified in EASD, encompassed several other genes.[15] • UF: no data on IP is available. However, this father had a history of hyperactivity and FH of epilepsy • VUS

[1](Namekata et al., 2019); [2](Griggs et al., 2008); [3](Krgovic et al., 2018); [4](Wang et al., 2016); [5](Manville and Abbott, 2019); [6](Miceli et al., 1993); [7](Miceli et al., 2015); [8](Piro et al., 2019); [9](Epi4K Consortium et al., 2013) [10](Sands et al., 2019); [11](Maljevic et al., 2016), [12](Wei et al., 2017); [13](Robertson et al., 2015); [14](Fu et al., 2020); [15](Reinthaler et al., 2014)

Case 78 (Classical LKS)

Case summary:

Case 78 is a boy who had a history of a transient floppy unresponsive episode at 8 months of age. This resolved spontaneously and was not further investigated. His speech was assessed by a speech and language therapist at 2.5 years of age and his skills were thought to be age appropriate. At 2.5 years of age, he had another short episode of unresponsiveness with cyanosis. 2-3 weeks later, he had his first GTCS. Since then, he developed frequent focal motor and generalised tonic clonic seizures. Neurometabolic investigations returned normal results. He was commenced on sodium valproate but continued to have frequent seizures. During this time, his speech became unintelligible and he had difficulty with balance. This was attributed to sodium valproate, and he was started on Levetiracetam. On Levetiracetam, his seizure burden reduced. His balance and speech also showed some signs of improvement, although he continued to have significant speech and language impairment. He became seizure free for 2 years on Levetiracetam, however, his seizures recurred after a sore-throat with some regression in his speech and language. At his last assessment, he fell within the borderline ID range for non-verbal abilities, and in the severe impairment range for language abilities. His AED dose was optimised and his parents were going to consider steroid therapy.

Early development: Normal

Co-morbidities: unbalanced gait, aggression, attention deficit hyperactivity disorder (treated with methylphenidate), mild ID

Family history: NIL

Dysmorphic features: NIL

Examination findings: Weight and height 91st centile, head circumference: 50th centile. Mild peripheral hypotonia, bilateral in-toeing. Otherwise normal

MRI: Normal

EEG: Bilateral independent centro-temporal epileptiform discharges with activation in sleep occupying approximately 32% of sleep record, not meeting criteria for ESES.

Genetic findings for Case 78

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ADGRV1 (UF) c.6931G>A p.D2311N	Moderate to high	Encodes a member of the adhesion family of G-protein coupled-receptors. Some members of this family – ADGRL1 have a role in axon guidance and synaptogenesis. ADGRV1 is found at synapses at cochlear and retinal cells and has been found to be needed for GABA-ergic interneuron development in the auditory cortex. It remains to be established if it may have a more widespread role in the rest of the CNS. [1,2]	• LGS, JME, EOAE, GE +/- ID[2]	• Gene function has possible link to LTP/Sz mechanisms • Some overlap in PDP and LKS- Sz, ID • Variants in this gene identified in 3 families within this LKS cohort	• All variants inherited from unaffected parents- however IP has been reported in relation to this gene. • All variants have been classified as VUS
LGALS3 (UM) c.484C>T p.R162C	Low	Encodes lectin, galactoside soluble protein-3, a member of the lectin and beta-galactoside binding family of proteins, with roles including cell-cell adhesion, macrophage activation and cell-matrix interaction. The exact role of this protein in the CNS is unclear but a study has reported elevated serum levels of this protein in refractory epilepsy compared to control. This gene is also upregulated in a pilocarpine induced TLE murine model. [3]	• EASD (CNV, MA)[4]	• One of the genes in a CNV region identified in an EASD patient • Function of gene suggests possible link to epilepsy	• Inherited from an unaffected mother, with no data available on IP. • Classified as a VUS • Only 1 case identified in this LKS cohort • Although previously described in an individual with EASD this was as part of a CNV, encompassing several other genes.

[1](Hamann et al., 2015); [2](Myers et al., 2018); [3](Tripathi et al., 2016); [4](Reinthaler et al., 2014)

Case 82 (Classical LKS)

Case summary:

A boy who presented to GOSH DEC clinic at 4 years 8 months of age. He had normal early development and was said to have been advanced with good bilingual language skills. At the age of 4 years 3 months, after a cold, his parents noted he would not respond to questions, then that he would start to say a sentence but not finish it, and act as though he was frustrated because he could not find the correct words. Over the course of 4 months, he became largely non-verbal and at times, lost the ability to comprehend environmental sounds. He did not have any overt clinical seizures and his first awake and sleep EEG done at this time was reported to be normal. Hearing assessments also returned normal results. 2 months later, although he continued to have no clinical seizures, a repeat EEG showed signs of ESES. He responded well to a 6-week course of oral prednisolone, with some recovery of his speech and language abilities. He did not, however, return to his baseline and his abilities continued to fluctuate. In addition, he continued to have significant difficulties with behaviour and attention. His EEG after prednisolone showed no epileptiform discharges. At his last assessment at 4 years 10 months, just after his course of steroids, he scored within the moderate impairment range for his language abilities.

Early development: Normal

Co-morbidities: behavioural difficulties, with oppositional behaviour, impulsivity and attentional difficulties, some ASD traits

Family history: History of bipolar disorder and adult onset Parkinson's disease in relatives (no details on maternal/paternal)

Dysmorphic features: NIL

Examination findings: Normal neurological examination. Weight: 50th centile, height: 25th centile, Head circumference: 25th centile

MRI: Normal

EEG: Right mid and fronto-temporal spike wave discharges. ESES in sleep.

Genetic findings for Case 82

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
IRX6 (*de novo*) c.1334C>A; p.A445E	Moderate to high	A homeobox gene encoding a transcription factor. The function of this gene is not yet well established. However, IRX-6 has been found to be involved in neurogenesis in murine models.[1]	• NIL	• Function of gene suggests possible link to mechanisms involved in LTP/Sz • This is a de novo variant that is absent from all population databases and has been classified as likely pathogenic by ACMG criteria	• Variants in this gene have not previously been identified in LKS, epilepsy or neurological disorders • Only 1 case in this LKS cohort
MYSM1 (*de novo*) c.1301G>A; p.R434H	Low	Encodes an enzyme with deubiquitinase catalytic activity. MYSM1 regulates gene regulation through deubiquitination of histone-2A and non-catalytic contacts with other transcriptional regulators. Its role is best characterized as an important regulator of haematopoiesis and immunity. Its role in the CNS has not yet been well-established.[2]	• NIL neurological- MYSM1 deficient patients have bone marrow failure syndrome. They may have NDD but NDD is not the predominant feature	• This is a *de novo* variant that is PTBP by most in silico databases. • Although this proband does not have an immunological phenotype, his symptoms started after a viral infection	• This gene's function is not well characterised within the CNS • Variants in this gene have not previously been identified in LKS, epilepsy or neurological disorders • Only 1 case in this LKS cohort

[1](Cohen et al., 2000); (Fiore et al., 2020)

Case 89 (Classical LKS)

Case summary:

A boy who presented at the age of 3.5 years of age with his first seizure – a prolonged GTCS. He had normal early development. In fact, he was bilingual and was reported to be advanced in language skills at 3.5 years of age. 2 months after his first seizure, his mother noted that he had lost his ability to count and that he had forgotten the alphabet. He did not lose speech completely but he had increasing word-finding difficulty and difficulty understanding instructions. At the time of review at GOSH DEC, he had ongoing seizures and language difficulties despite AED treatment. In addition to GTCS, he had focal motor seizures and absence seizures. He had very good response to steroid therapy with seizure cessation and marked improvement in his language skills. After steroid therapy stopped, his seizures recurred, but at his last assessment at 7 years of age, his assessments demonstrated ongoing improvement with speech and language skills.

Early development: Normal

Co-morbidities: Motor dyspraxia, coordination difficulties, anxiety, autistic traits, attention difficulties

Family history: NIL significant

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal

EEG: left and right centro-temporal discharges with right predominance, ESES in sleep

Genetic findings for Case 89

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
GABBR2 (*de novo*)	High **Top candidate gene for this proband**	Encodes subunit 2 of the gamma-amino-butyric acid Type B ($GABA_B$) receptor which mediates slow synaptic inhibition within the brain.[1]	• EIEE-59 (MA)[2] • NPLHS (MA)[3]	• Gene function links to Sz mechanisms • Phenotype overlap between PDP and LKS: regression, SLI, Sz, ASD • De novo variant absent in all PD • Classified as pathogenic	• Not previously identified in LKS and only 1 case in this LKS cohort.
PAX6 (APS) c.1304T>A p.L435*	Low to moderate	Encodes paired box gene 6, a transcription factor integral to the development of many tissues including the brain. In addition to its role in neuro-development, PAX6 is also thought to have important roles in the maintenance of brain parenchyma particularly after injury.[4] *PAX6* haploinsufficiency has been shown to lead to impaired hippocampus synaptic plasticity.[5]	• CECTS (MA/BA)[6] • Aniridia, hyposmia, cortical thinning abnormal cortical pattern and working memory deficits (MA)[4,7]	• Function of gene has possible links to mechanisms for sz and memory • Significant phenotype overlap between CECTS and LKS	• Reported link to CECTS[2] is through a specific variation in the 3'UTR of *PAX6,* rs662702, that has not been found in this LKS cohort. • The functional effect of rs662702 was proposed to increase expression of *PAX6,* which is not likely to occur with this stop mutation[5] • Only 1 case in this LKS cohort

[1](Burmakina et al., 2014); [2](EuroEPINOMICS-RES Consortium et al., 2014); [3](Vuillaume et al., 2018); [4](Yogarajah et al., 2016) ; [5](Callaerts-Vegh, 2009); [6](Panjwani et al., 2016); [7](Lipponen et al., 2018)

8.4 *GRIN2A*-negative atypical cases

Case 16 (Atypical- no clear history of regression)

Case summary:

Case 16 is a boy who presented to GOSH DEC at 8 years 2 months of age. He had early motor and speech delay. He did not sit until the age of 9 months and started walking at 18 months of age. He said his first words "mum", "dad" and "bar" at the age of 3 years. He had a febrile seizure at 3 years 1 month of age. A month after this, he had his first afebrile GTCS. He proceeded to have relatively frequent seizures of various semiology including drop attacks, absence seizures and myoclonic seizures. He continued to have severe speech and language difficulties with some fluctuation but there was no clear history of regression. He began to rely largely on sign language. As he got older, he had progressively worse motor difficulties, with dystonia, dyspraxia and myoclonic jerks particularly affecting his left side. In his teenage years, he started having increasing difficulty walking and used a walking frame. As an adult, he lives in a residential home and continues to have frequent seizures.

Early development: Speech and language delay: 1st words at 3 years. Motor delay – walked at 18 months,

Co-morbidities: Motor difficulties: dystonia and myoclonic jerks. Had difficulty walking as illness progressed. Attention difficulties. Behavioural difficulties with tantrums and aggression, autistic traits.

Family history: Maternal grandmother had refractory epilepsy from 16 years of age

Dysmorphic features: NIL

Examination findings: High stepping gait on his left, with occasional myoclonic jerks in the left leg

MRI: Normal

EEG: Sharp waves over left or right centrotemporal region, ESES in sleep

Genetic findings for Case 16

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against
MYH7B (APS) c.2433+5G>A	Moderate to high	Encodes myosin heavy chain 7B, an actin binding protein that has been shown to have a role in maintaining excitatory synaptic function by regulating dendritic spine structure and AMPAR trafficking in the hippocampus.[1]	• NIL neurological	• Gene function links to LTP/Sz mechanisms • Variants in this gene were identified in 4 families within this LKS cohort	• Some variants identified in this cohort, were inherited from unaffected parents, no data on IP available

[11](Rubio et al., 2011)

Case 30 (Atypical – no clear history of regression)

Case summary:

Case 30 is a boy who had onset of focal motor seizures and GTCS at the age of 2 years 1 month. Some of his seizures were prolonged and associated with post-ictal slurred speech and unsteady gait. He had a long-standing history of speech and language delay, but there was no history of clear regression. At presentation at GOSH DEC at 4 years 3 months of age, he was speaking in short sentences with poor articulation and understanding 2 stage commands. He had poor hand coordination and oromotor dyspraxia affecting speech and feeding. He had a course of steroid therapy after which there was some improvement in his speech, his behaviour and motor skills. He was supported with regular OT and SLT, and made gradual improvement. At his last review, at the age of 14 years, he was seizure free allowing gradual withdrawal of his AEDs. His speech and language abilities were estimated to be at the age-equivalent range of 3 years 2 months to 5 years 0 months (severe impairment), and his non-verbal skills were estimated to be at a 6-year age-equivalent level. He continued to have significant behavioural difficulties.

Early development: Speech and language delay

Co-morbidities:

ADHD, ASD, some fine motor difficulties and motor dyspraxia – unable to jump or hop at 6 years of age, behavioural difficulties – aggression, ID

Family history: Mother's cousin had a history of epilepsy, DD, and poor coordination as a child, 2 cousins with ASD (not mentioned if paternal or maternal)

Dysmorphic features: NIL

Examination findings: Head circumference 2^{nd} to 9^{th} centile, Height: 2^{nd} to 9^{th} centile, Weight: 0.4^{th} centile (13 years old)
Some signs of occasional drooling, motor dyspraxia and joint hypermobility. Otherwise within normal limits.

MRI: Normal at 4 years

EEG: Frequent focal discharges over either left or right centro-parietal regions (different laterality on separate EEGs) with activation in sleep to the point of being almost continuous, ESES.

Genetic findings for Case 30

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
WDFY3 (*de novo)* c.2866delG; p.D956fs*5	Moderate/ **High** **Top candidate gene for this proband**	Encodes WD40-repeat, FYVE Domain containing protein. It is thought to have a role in autophagy and may have an important role in axon guidance, establishing neuronal connections and neuronal migration in the developing brain.[1-3]	• NDD with miC or maC, ID, ASD, ADHD, SLI (MA)[4,5]	• Function of gene may link to mechanisms important for LTP/Sz • *De novo* protein truncating variant in a gene where loss of function variants have been identified in similar phenotype – SLI, ASD, ADHD, ID • Classified as pathogenic	• This proband does not have miC/maC although this is not present in all patients with variants in this gene[5] • Not previously described in Sz/LKS phenotypes • Only 1 case in this LKS cohort who does not have typical LKS
NLRP3 (*de novo*) c.86T>C p.L29S	Moderate/ Moderate	Encodes NOD-like receptor protein-3, part of an inflammasome complex that stimulates the release of inflammatory factors like Il-1B. The NLRP3 inflammasome has been implicated in epileptogenesis, and epileptic neuronal apoptosis.[6-8]	• AIS without predominant neurological symptoms (MA)[8]	• Gene function linked to epileptogenesis • This is a de novo variant that is absent in all PDB, PTBP by most in silico prediction algorithms, and classified as likely pathogenic	• This proband does not have symptoms of AIS. • Variants in this gene have not previously been reported in Sz/LKS phenotypes • Only 1 case in this LKS cohort and this proband does not have typical LKS
ARHGEF4 (UF) c.854G>A p.R285Q	Moderate/ Moderate	A Rho- guanine nucleotide exchange factor, that inhibits synaptic localization of post-synaptic density- 95 (PSD-95), a major scaffolding protein in the post-synaptic density. By acting as a specific guanine-exchange factor, for the GTPase CDC-42 (cell-division cycle-42), ARHGEF4 has also been found to stimulate dendritic outgrowth.[9]	• CECTS (only gene within a CNV, MA)[10] • DEE with ID, SLI, ADHD (recurrent CNV hotspot in 2q21.1 with 5 genes)[11]	• Gene function links to LTP/Sz • Significant overlap between LKS and previously reported phenotypes – CTS, SLI, ADHD • Identified in 2 individuals within this LKS cohort • Both rare mutations that are PTBP–this one lies within the Dbl homology domain essential for GEF activity, the other is a PTV,	• Inherited from unaffected father. No available data on IP. However, there is a positive FH. • Both variants have been classified as VUS

[1](Dragich et al., 2016b); [2](Napoli et al., 2018b); 3(Orosco et al., 2014), [4](Guo et al., 2018); [5](Le Duc et al., 2019); [6](Shen et al., 2018), [7](Wu et al., 2019), [8](Eren and Özören, 2019); [9](Oh et al., 2018), [10](Addis et al., 2018); [11](Dharmadhikari et al., 2012)

Case 43 (Atypical – no clear regression)

Case summary:

Case 43 is a boy who was noted to have speech delay from 2 years of age. Other developmental milestones were within normal limits. Frequent focal impaired awareness seizures commenced from the age of 3 years. Other seizure types included absence seizures. His seizures were controlled with anti-epileptics with some improvement in his language. There has never been a history of regression. Over repeated assessments and review, he has continued to make some progress with his language skills, although at his last language assessment at 14 years of age, he continued to score within the severe impairment range.

Early development: Speech delay

Co-morbidities: moderate intellectual disability, difficulties with attention and impulsivity, social communication difficulties- not meeting criteria for ASD

Family history: Maternal uncle had seizures as a baby

Dysmorphic features: NIL specific. Possible mild micrognathia, prominent mandible, broad tip of nose

Examination findings: HC: 2nd to 9th centile. Joint hyperlaxity noted. Otherwise, no significant abnormalities

MRI: Normal at 6 years of age

EEG: Abnormal background with bifrontal and asynchronous central spikes. Focal ESES in temporal regions.

Genetic findings for Case 43

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function/Notes:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
Chromosome 1p duplication 1p36.33p.36.32 (751796-4678966)x3 ish der(14)t(1;14) (p.36.32;q32.33) (*de novo)*	Low/High	Discovered via microarray study performed at referring diagnostic laboratory Pathogenic genes encompassed include: *AGRN, ATAD3, GABRD, TMEM240, CEP104, GNB1, PEX10, SKI, DVL1, B3GALT6, TP73, PRKCZ, KLHL17, PRDM16*[1]	• NDD with DysF, MiC, MaC; Sz, ID, BD, cardiac defects, SkAbn[1]	• This proband has some DysF, ID, BD, Sz consistent with PDP	• This proband does not have many features of PDP – cardiac defects, SkAbn • Such CNVs have not previously been reported LKS. • Only 1 case in this cohort and this case does not have classical LKS.
SOCS7 (*de novo*) c.1609G>C; p.D537H	Moderate/ moderate to high	Encodes suppressor of cytokine signalling protein 7, which inhibits cytokine signalling by linking signalling molecules to E3-ubiquitin ligases[1]. SOCS7 interferes with the reelin signalling pathway through degradation of one of its effector proteins DAB1 (disabled-1)[2]. This pathway is important for neuronal cytoarchitecture and for postnatal dendritic growth and synaptic plasticity. BA mutations in reelin result in lissencephaly[3], whilst MA mutations have been a/w TLE with normal MRI[4].	• NIL neurological	• Function of the gene may have links to LTP/Sz mechanisms • DNV that is absent in all PD and is classified as likely pathogenic. Lies in SOCS box domain vital for recruitment of E3 ubiquitin-ligase complex.	• Variants in this gene have not previously been reported in epilepsy/LKS. • Only 1 case in this cohort and this individual does not have classical LKS.
PLEKHG2 (CH- biparental) c.2284C>T; p.R762C c.2465G>A; p.R822Q	Low / Moderate	Encodes a Rho- guanidine exchange factor (GEF) which may have a role in actin cytoskeleton arrangement, neuronal network formation, dendritic arborization and spine formation[5].	• ID, dystonia, miC, and WM abN (BA)[5]	• Function of the gene may have links to important mechanisms involved in LTP and Sz • Both variants are rare and PTBP	• Variants in this gene have not previously been reported in epilepsy/LKS. • Only 1 case in this cohort and this individual does not have classical LKS. • Apart from ID, the PDP is quite distinct from LKS

[1](Marquet et al., 2017); [2](Lawrenson et al., 2017); [3](Hong et al., 2000); [4](Dazzo et al., 2015); [5](Edvardson et al., 2016)

Case 50 (Atypical due to global regression of skills)

Case summary:

Case 50 is a girl who presented to GOSH DEC at 10 years of age. She had a history of congenital nystagmus, short stature and mild motor delay but early speech and language development was within normal limits. Learning difficulties were identified at Year 2 of school. She developed seizures at 9 years of age including GTCS, tonic, atonic and myoclonic seizures. From the age of 9 years 11 months, she had gradual global regression in developmental abilities, including language, cognition and motor skills. At her worst, she was non-verbal with severe impairment of non-verbal cognitive skills and she had so much difficulty with tremor and movement coordination that she was dependent on a wheelchair for 2 years. She had partial response to steroid therapy but she developed hypertension and hyperglycaemia so this had to be stopped. Her seizures proved refractory to several anti-epileptics, but her seizure burden was significantly reduced with the ketogenic diet. With control of her seizures, she had some return of her motor skills, and was able to ride a bicycle. She, however, remained non-verbal and communicated largely using an IPad with symbols. At the age of 20 years, she was being educated at a local college and continued to live with her parents. She was off all anti-epileptic medication but her parents reported that she may have a rare seizure.

Early development: Mild motor delay: stood at 13 months, cruised at 15 months and walked at 20 months of age. Normal speech and language development

Co-morbidities: Cognitive impairment, significant motor difficulties including, dyspraxia, ataxia and tremor.

Family history: All siblings have short stature, developmental delay and congenital nystagmus. Both parents are healthy

Dysmorphic features: NIL

Examination findings: Normal tone, power and reflexes, action tremor, dyspraxia. Short stature. Weight: 0.4th centile, height:0.4th to 2nd centile, head circumference: 2nd to 9th centile.

MRI: Normal

EEG: Bilateral independent centro-temporal discharges and intermittent generalized bursts. ESES in sleep.

Genetic findings for Case 50

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
VPS13A (BP) c.5587G>A; p.A1863T and c.2888C>T; p.T963M	Low to moderate/ Moderate	Encodes chorein, a protein which binds to phosphatidylinositol lipids on cell membranes and interacts with cytoskeletal proteins. Its many functions including maintenance of cytoskeletal architecture, vesicular trafficking, neurotransmitter release, autophagy, neuronal cell survival and mitochondrial function.[1]	• Chorea-acanthocytosis (BA)[1] • TLE without movement disorder (BA)[2]	• Gene function can possibly linked to LTP/Sz mechanisms • Both variants rare and PTBP • Some phenotypic overlap between PDP and this proband– focal Sz, motor difficulties	• For A.D., symptom onset is usually in adulthood • Single case within this cohort, not previously associated with LKS and this proband does not have typical LKS
LAMA5 (UM) c.4879C>T p.R1627C	Moderate/ Moderate	Encodes laminin alpha-5, an extracellular matrix glycoprotein with a role in the regulation of dendritic spine density and synaptic stability in the hippocampus[3]	• SLI, DD, ASD, Sz (MA)[4]	• Gene function linked with LTP and Sz mechanisms • Some overlap in PDP and LKS – SLI, ASD, Sz • Variants in this gene identified in 3 families within this LKS cohort	• Variant in this gene previously reported in only 1 child with SLI, DD, ASD, Sz. Not previously reported in LKS • Some variants in this cohort were inherited from unaffected parents, no data on IP available.

[1](Lang et al., 2017); [2](Weber et al., 2018); [3](Omar et al., 2017); [4](Han et al., 2018)

Case 52 (Atypical- no clear history of regression)

Case summary:

Case 52 is a boy who presented to GOSH DEC at 8 years 5 months of age. He had a long-standing history of speech and language impairment. His parents reported that at approximately one year of age, he would largely communicate through grunts and pointing. As he got older, he learnt a few single words but rarely used them. He learnt sign language at 4 years of age, and since then has used this as his main means of communication. There was no history of developmental regression. He developed seizures at the age of 3 years. These have been characterised by behavioural arrest, being unresponsive, drooling then losing tone. His EEG showed signs of left sided ESES, and before presenting at GOSH DEC, he had been tried on multiple AEDs, and a trial of steroids. These were effective in controlling his seizures but had limited effect on his language abilities. At 8 years 5 months of age, developmental assessment found that he had average non-verbal abilities, but severe speech and language impairment. His verbal comprehension was at the one stage command level, and he struggled to manage even one-word expression with poor oromotor planning. At times he would use consistent sounds to mean certain things, but these would not be understood by an unfamiliar person. Throughout follow up he continued to be seizure free but continued to have severe speech and language impairment.

Early development: Speech and language impairment

Co-morbidities: Autistic traits – some rigid behaviour, obsessions and stereotypies

Family history: NIL significant

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Interval left hippocampal increased signal and loss of volume at 6 years 11 months of age

EEG: Left centro-temporal spikes which became continuous in sleep but did not generalise

Genetic findings for Case 52

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
ADGRV1 (UF) c.13850G>A p.G4617E	Moderate to high	Encodes a member of the adhesion family of G-protein coupled-receptors. Some members of this family – ADGRL1 have a role in axon guidance and synaptogenesis. ADGRV1 is found at synapses at cochlear and retinal cells and has been found to be needed for GABA-ergic interneuron development in the auditory cortex. It remains to be established if it may have a more widespread role in the rest of the CNS. [1,2]	• LGS, JME, EOAE, GE +/- ID[2]	• Gene function has possible link to LTP/Sz mechanisms • Some overlap in PDP and LKS-Sz, ID • Variants in this gene identified in 3 families within this LKS cohort	• All variants inherited from unaffected parents- however IP has been reported in relation to this gene. • All variants have been classified as VUS
ADAMTSL4 (BP) c.2848G>C; p.G950R c.190G>A; p.V64M	Low/Low	Encodes A-Disintegrin and Metalloproteinase with Thrombospondin Motif Like-Protein 4 Its function is not completely understood but it is related to fibrillin-1 and is likely to have a role in extracellular matrix homeostasis.[3]	• Ectopia lentis (BA)[3]	• Compound heterozygous variants that are rare and PTBP by most in silico prediction algorithms	• Function of gene within the CNS is not clear • Not previously described in LKS/neurological disorders • Only case within this cohort, and this proband does not have typical LKS

[1](Hamann et al., 2015); [2](Myers et al., 2018);[3](Le Goff and Cormier-Daire, 2011)

Case 57 (Atypical as no clear history of regression)

Case summary:

Case 57 is a boy who was referred to GOSH DEC at 6 years of age. He had a history of early speech and language delay. His first seizure occurred at the age of 3 years and was characterized by up-rolling of his eyes, loss of tone and cyanosis. He had 2 episodes on the same day and was started on sodium valproate. This did not adequately control his seizures, and he developed other seizure types including absence seizures and focal impaired awareness seizures with vomiting and hypotonia. He eventually achieved seizure control when Levetiracetam was added on as adjunctive treatment. His parents felt his speech improved with seizure control, but his EEG continued to show signs of ESES. There was no history of developmental regression. On assessment at GOSH DEC, his non-verbal skills fell within the low average range but he scored within the severe impairment range for his language skills. He was supported with a statement of education, and on repeat assessments, made steady progress. At his last assessment at 10 years of age, he was assessed to be at the age equivalent of approximately 5 years for his language assessment and within the borderline range for his non-verbal skills. He was seizure free although he continued to have sleep activation of epileptiform discharges of up to 60% SWI on EEG.

Early development: Speech and language delay

Co-morbidities: Fine motor difficulties

Family history: Father had a history of febrile seizures

Dysmorphic features: NIL

Examination findings: Weight: 75th centile, head circumference: 75th centile, normal neurological examination.

MRI: Normal

EEG: Sharp and slow wave complexes over bilateral centrotemporal regions right > left. ESES in sleep.

Genetic findings for Case 57

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
RORB (*de novo)* c.896G>A; p.C299Y	Moderate to high /High Top candidate gene for this proband	Encodes retinoid related orphan receptor B, a ligand dependent transcription factor. Its roles in the CNS include transcriptional control of neuronal differentiation, neurogenesis and thalamocortical axon guidance.[1-3]	• FE/GE/DEE, many with SLI, ID, DD (MA)[3,4]	• Function of gene suggests link to LTP and Sz mechanisms • Overlap between LKS and PDP: SLI, DD, Sz • DNV that lies within the important LBD of this protein, is PTBP by most in silico algorithms, is absent in all PDB and is classified as likely pathogenic	• Not previously described in LKS • Only case in this cohort and this child does not have classical LKS.
LIMD1 (*de novo)* c.87C>A; p.F29L	Low to moderate/ Moderate	Encodes LIM-domain containing protein 1, a transcription regulator. It is involved in the regulation of cell adhesion, cytoskeletal organisation, cell morphology and cell migration. Its function in the CNS is not well characterised.[5]	• FE with DD (CNV/MA)[6] – 1 case • TLE with aphasic Sz[7] (MA) – 1 family	• Isolated reports of variants in focal epilepsy with some phenotypic overlap with LKS – FS, DD, aphasia • DNV that is PTBP by many in silico algorithms and classified as likely pathogenic	• Not previously described in LKS • Only case in this cohort and this child does not have classical LKS • Function in the CNS is not well-characterised.
PHF20 *(de novo)* c.146G>A; p.R49H	Low to moderate/ Low to moderate	Encodes plant homeodomain finger 20, a protein with roles in transcriptional regulation and DNA repair. In theory, by associating with the H3K4- specific methyltransferase, MLL1, it may be implicated in the regulation of genes that are involved in neurogenesis, and synaptic development pathways.[8,9]	• NIL neurological	• DNV that is predicted to pathogenic by many in silico algorithms • May have theoretical links to LTP/Sz mechanisms but this has not been demonstrated	• Not previously described in LKS, • Only case in this cohort and this child does not have classical LKS • Classified as VUS

[1](Liu et al., 2017a); [2](Byun et al., 2019); [3](Rudolf et al., 2016); [4](Sadleir et al., 2020); [5](Bai et al., 2011) [6](Howell et al., 2013), [7](Dazzo et al., 2015); [8](Klein et al., 2016), [9](Kerimoglu et al., 2017)

Case 58 (atypical due to more global recession)

Case summary:

Case 58 is a boy who was admitted to the neonatal unit at birth for 1 week for feeding difficulties following thick meconium at delivery. His parents reported that at 9 months he had started saying “mama”, “dada” and “baba”, but he fell silent about 1 month later. At 1.5 years of age, he regained the ability to say some single words. At 3- 4 years of age, it was reported that he had a few single words and was communicating well using PECS and Makaton at a special learning needs nursery. Just before he turned 4, he had his first seizure. He had frequent seizures including focal motor seizures, atonic seizures, absence seizures and myoclonic jerks. From 4.5 years of age, he also had periods of non-convulsive status epilepticus, lasting for days, clinically characterised by drooling, dysphagia, and unsteady gait. Neurometabolic investigations proved inconclusive. He received treatment with multiple AEDs all of which had limited benefit. There was some evidence of steroid responsiveness and he had serial courses of prednisolone. By 5 years of age, he had gradually lost more skills, he stopped speaking and stopped gesturing, he stopped using his right hand then eventually stopped walking. There was evidence of dystonic posturing especially of his right hand on examination. At his last assessment at 8 years 11 months, he had variable seizure control, severe global developmental delay, and was completely dependent on care for all his activities of daily living.

Early development: Gross motor delay -- crawled at 11 months of age and did not walk independently until 23 months of age.

Co-morbidities: ID, motor dyspraxia, dystonia, ataxia, feeding difficulties, motor-stereotypies and automatisms- hand wringing, flapping

Family history: Maternal uncle has learning difficulties, paternal uncle had epilepsy (may be secondary to head injury)

Dysmorphic features: Broad nasal bridge, broad hand and tapered fingers, pectus excavatum, hypertrichosis on the back

Examination findings: Weight 2nd centile, height: 0.4th centile; head circumference: 0.4th centile. Hypotonia and ligament laxity, right divergent squint. At later stages: dystonic posturing, limited hand function and ataxic gait.

MRI: Generalised atrophy of the cerebrum and cerebellum (non-progressive)

EEG: Frequent discharges max over left centroparietal region and independently over the right hemisphere. Sleep activation occupying up to 60-70% of the sleep record.

Genetic findings for Case 58

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	**Against:**
KMT2A (*de novo)* c.3460C>T; p.R1154W	Low to moderate/**High** Top candidate gene for this proband	Encodes a histone methyltransferase enzyme that has a key role in gene expression regulation during development. Within the CNS, it has been shown that genes regulated by KMT2A are involved in key pathways for neurogenesis, synaptic development and neuroplasticity.[1,2]	• Wiedemann-Steiner syndrome (WDSS) (MA)[3] • NDD with ID, DF, SS, HT, GDD, +/- ASD +/- Sz • ASD (MA)[4] • DEE (including 1 patient with ESES and FE)[5]	• This proband has features of WDSS – SS, HT, GDD, ID, FD • De novo variant that is absent from all normal population databases and that is located within the zinc finger domain where missense variants for WDSS cluster – classified as likely pathogenic by ACMG • This variant has been previously identified in another patient with WDSS with Sz[6] • This variant has been functionally investigated and been found to alter KMT2A gene expression.[6] • This variant is listed on ClinVar (likely pathogenic) • Function of gene links to mechanisms important for LTP/ Sz	• Variants in this gene have not previously been described in EASD/LKS • Only case in this cohort and this proband does not have typical LKS – his phenotype may fit better with WDSS.

[1](Kerimoglu et al., 2017), [2](Vallianatos and Iwase, 2015); [3](Chan et al., 2019), [4](Li and Pozzo-Miller, 2019), [5](Helbig et al., 2016); [6](Lebrun et al., 2018)

Case 60 (Atypical- no clear history of regression)

Case summary:

Case 60 is a girl who had speech and language delay recognized from her early years. She said her first words at 18 months and her speech was always described as unclear. At presentation at GOSH DEC at 6 years of age, she was speaking in short phrases of 3-5 words and had difficulty with articulation. She made slow progress with her speech and language and there was no history of regression. Between the ages of 2 and 6 years, she had 4 febrile seizures, but she has not had a history of unprovoked clinical seizures. Her EEG showed very frequent discharges with sleep activation, occupying up to 81% of the sleep record. She was started on sodium valproate then ethosuximide, then had a course of prednisolone, but she continued to have sleep discharges approaching ESES on EEG. At her last review at 11 years of age, she continued to have severe speech and language impairment and epileptiform discharges occupying 50-75% of her sleep EEG record.

Early development: Speech and language delay, otherwise normal

Co-morbidities: NIL significant

Family history: Father had recurrent syncope at 13 years of age which spontaneously resolved. Mother had a history of post-traumatic seizures post RTA at 17 years of age.

Dysmorphic features: NIL

Examination findings: Head circumference :9th centile, weight and height: 50th centile, normal neurological examination

MRI: A few non-specific white-matter abnormalities on

EEG: Isolated high amplitude epileptiform discharges over the left sylvian region and independent discharges over the right sylvian and posterior temporal regions. Clusters of generalised spike/sharp and slow complexes of irregular frequency were noted, which during sleep, occupied up to 81% of the trace (varied from 30% to 81% across different recordings).

Genetic findings for Case 60

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
GRIP1 (UM) c.446G>A p.R149Q	Moderate/ Moderate	Encodes glutamate receptor-interacting protein 1, a synaptic scaffold protein with a well-established role in the stabilization and recycling of AMPA receptor subunits, it mediates synaptic plasticity mechanisms underlying learning and memory.[1,2]	• Fraser Syndrome (BA)[3] • Autism (MA/BA)[4] • LKS (CNV, MA)[5] • CECTS with SLI and MD (CNV, MA)[6] - inherited from mother with ADHD	• Function of this gene strongly links to mechanisms important for Sz and LTP • Variants previously described in LKS and in CECTS/ASD – phenotypes with significant overlap with LKS	• Inherited from unaffected mother. No clear data on incomplete penetrance available, but there are isolated reports of phenotypic variability[35,65] • Classified as VUS

[1](Bissen et al., 2019); [2](Trotman et al., 2014); [3](Schanze et al., 2014); [4](Mejias et al., 2011); [5](Conroy et al., 2014); [6](Addis et al., 2018)

Case 64 (Atypical – no clear regression)

Case summary:

Case 64 had his first seizure at the age of 10 years – mainly focal motor seizures characterized by jerking of his limbs on either side, at times with post-ictal aphasia. There was a history of delayed speech and language acquisition noted at the age of 2 years, but there was no history of clear regression. An EEG at presentation showed signs of ESES. Neuropsychological testing and speech and language testing demonstrated language and cognitive skills in the low average range. His examination was notable for inversion of feet during walking, and at this time an electromyogram showed some signs of myopathy. His creatine kinase level was within normal limits. He was referred to the neuromuscular service and was not felt to have primary neuromuscular disease as he had normal muscle strength and a normal muscle ultrasound. He is athletic and enjoys sports. At last assessment at 14 years of age, he was seizure free, and was being educated at an independent school with small classes and a statement of special educational needs. There has been some concern regarding difficulty with attention and hyperactivity, but he has not been formally diagnosed with ADHD.

Early development: Speech delay noted from 2 years of age. Other development areas within normal limits.

Co-morbidities: Attention difficulties, hyperactivity, impulsivity

Family history: Paternal first cousin had seizures as a baby (attributed to hypocalcaemia)

Dysmorphic features: NIL

Examination findings: Normal anthropometry, weight and HC: 75th centile. Normal neurological examination, apart from scattered café au lait patches and tendency to walk with bilateral feet inverted

MRI: Normal at 10 years

EEG: Bilateral independent centrotemporal spikes, ESES

Genetic findings for Case 64

Gene (Inheritance)/ Variant:	Likelihood of Significance for LKS/Proband:-	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
RYR3 (AUM) c.10067G>T; p.R3356L	Moderate	Encodes ryanodine receptor type 3 with a role for releasing calcium from intracellular stores in the ER[1]. This calcium release has an important role to play in synaptic plasticity and memory formation[2].	• WS (MA/BA)[1,3] • EIEE with ID, SLI, BD(MA)[3] • NM (BA)[4]	Gene function can be linked with Sz/LTP mechanisms. • Overlap in PDP and LKS: Sz, ID, SLI, ASD • May contribute to proband's myopathic EMG	• PDP had younger age of onset of Sz than this proband (6m to 8.5m vs 10 years) • Only 1 case within this cohort, not previously associated with LKS • VUS
GABRB1 (UM) c.1232G>T; p.R411L	Moderate	Encodes the beta-1 subunit of the $GABA_A$, a pentameric channel that mediates fast inhibitory synaptic transmission in the CNS[5].	• WS[6], EOEE[7], EIMFS[8] (MA) • ASD[9] (MA)	• Gene function can be linked with seizures Some overlap in previously associated phenotypes and LKS – seizures, DD	• Inherited from unaffected mother no available data on IP • Only 1 case in this LKS cohort, not previously associated with LKS
BSN (UM) c.4441T>A p.S1481T	Moderate to high	A component of the pre-synaptic cytomatrix, with a role in cytomatrix organization for neurotransmitter release[10] maturation of glutamatergic synapses[11] and neuroplasticity[12]	• LKS[13]	• Function of gene links to Sz and LTP mechanisms • Previously reported in 2 other unrelated patients with LKS[13] all variants occur in the same domain*	• Inherited from unaffected mother, no data available on IP • Classified as VUS
EPHB2 (AUM) c.3082_3083 delGA p.D1028fs*13	Moderate to high	A tyrosine kinase receptor with roles in the function and plasticity of excitatory synapses. EPHB2 mediates memory recall in the auditory cortex and hippocampus.[15]	• LKS (MA)[13]	• Function of gene links to Sz and LTP • Previously reported in 2 unrelated patients with LKS[13]	• Classified as VUS
MYH7B (AUM) c.5303delA p.Q1768fs*6	Moderate to high	Encodes an actin binding protein involved in regulating dendritic spine structure and AMPAR trafficking in the hippocampus.[16]	• NIL neurological	• Gene function links to LTP/Sz • Variants identified in 4 families in this LKS cohort	• Some variants identified were inherited from unaffected parents, no data on IP available

[1](Peng et al., 2018); [2](Balschun et al., 1999) [3](EuroEPINOMICS-RES Consortium et al., 2014); [4](Nilipour et al., 2018); [5](Hernandez and Macdonald, 2019); [6](Epi4K Consortium et al., 2013); [7](Lien et al., 2016); [8](Burgess et al., 2019); [9](Collins et al., 2006); [10](tom Dieck et al., 1998); [11](Lanore et al., 2010); [12](Ivanova et al., 2016); [13](Conroy et al., 2014); [14](Altrock et al., 2003); [15](Talebian and Henkemeyer, 2019); [16](Rubio et al., 2011); * p.S1481T has previously been reported in another individual with LKS with unknown inheritance. A variant at the neighbouring a.a., p.P1482L was reported in another unrelated LKS patient.[13] All these variants occur in exon 5, demonstrated in functional studies to be important for anchoring of BSN to the cytomatrix active zone.[14.]

Case 75 (Atypical)

Case summary:

This boy presented at the age of 10 years with severe behavioural difficulties, and GTCS and focal motor seizures from 11 years of age. There was no clear history of a speech/language regression. His EEG showed signs of ESES. His older brother had a history of classical LKS*. The older brother presented at 19 months of age years with speech and language difficulties and significant behavioural difficulties. He regressed from speaking 2 to 3-word phrases at 18 months of age to gradually losing expressive speech over the course of 4 weeks at 19 months of age. Receptive language skills also deteriorated in the next 2 months. He was noted to have some myoclonic jerks in sleep and some day time twitching of his eyes and nose but no clear history of seizures. Both brothers had some improvement with steroid therapy.

Early development: Normal

Co-morbidities: Behavioural difficulties

Family history: Apart from these 2 brothers, no other family members were reported to have epilepsy or learning difficulties.

Dysmorphic features: NIL

Examination findings: Normal neurological examination

MRI: Normal. Older brother's MRI at 2 years of age showed: a solitary white matter lesion in the left frontal lobe that was non-specific and not felt to be related to his presenting symptoms.

EEG: Centrotemporal discharges activating in sleep, meeting criteria for ESES. Older brother: right central discharges increasing with drowsiness and sleep, not meeting criteria for ESES.

*****Older brother's DNA was unfortunately not available for testing.

Genetic Findings for Case 75

Gene (Inheritance)/ Variant:	Significance for LKS/Proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
SETD5 (UF) c.2462G>A; p.C821Y	Moderate/ Moderate	Encodes SET [Su(var)3-9, enhancer of zeste, trithorax] domain containing protein 5, a domain that typically has a role in histone methyltransferase activity. It has been demonstrated that the epigenetic processes mediated by SETD5 regulate gene expression processes that affect neural stem cell proliferation, synapse formation and glutamatergic transmission[1]	• WS with hand stereotypies (MA)[2] • ASD, ID, facial dysmorphism (MA)[3,4]	• Gene function can be linked with seizures and LTP. • Some overlap in previously associated phenotypes and LKS – including seizures, DD, ASD, MD • 2 cases in this cohort (same variant identified in case 86- yet to be verified)	• Inherited from unaffected father and variant reported (albeit found to be rare) on EVS and gnomAD. However, incomplete penetrance has been reported. • VUS
FOXP2 (UM) c.735G>C; p.Q245H Awaiting Sanger confirmation	Moderate/ moderate	Encodes Foxhead box P protein 2, a member of a family of transcription factors. FOXP2 is highly expressed in the CNS, and is thought to coordinate pathways important for brain development and function, including synaptogenesis[5,6]. Human brain imaging studies have shown that *FOXP2* mutations alter the structure and function of the cortex, BG and cerebellum.[5]	• NDD with SLI, ASD, ID[5] • ECSWS[7]	• Gene function links with LTP and SLI processes • Significant overlap of clinical features for PDP and LKS: EASD, SLI, ASD • 2 cases within this LKS cohort (3.7%) and 2 cases of ECSWS within Lesca et al's similar cohort of LKS/ECSWS patients (3.2%)	• UM • This proband did not have as much of a SLI as his older brother

[1](Sessa et al., 2019); [2](Kobayashi et al., 2016); [3](Fernandes et al., 2018); [4](Powis et al., 2018); [5](Co et al., 2020); [6](Sia et al., 2013); [7](Lesca et al., 2012)

Case 81 (Atypical)

Case summary:

Case 81 is a boy who presented to GOSH DEC at 7 years 11 months of age. His early development was reported to be normal. At 2 years of age, he was saying up to 2-word phrases in Punjabi. However, after this time, his parents noted that his speech and behaviour deteriorated significantly. He used his own neologisms and became aggressive, biting and hitting others. He was diagnosed with autism spectrum disorder at 7 years of age. There was no history of clinical seizures. Assessment at GOSH DEC showed severe learning difficulties and speech and language impairment. His EEG was not typical of EASD, but showed some mild activation in sleep. He had a 6-week trial of steroid therapy, after which there was no objective improvement in his cognitive or verbal abilities, so this was weaned off. Overall, it was felt that his clinical picture fitted better with ASD regression than with LKS.

Early development: Within normal limits

Co-morbidities: ASD, ADHD, behavioural difficulties - aggression

Family history: Mother had 1 previous miscarriage. NIL else significant. He had 2 younger siblings who were healthy.

Dysmorphic features: NIL

Examination findings: Head circumference 0.4th to 2nd centile, Weight: 9th centile, Height

MRI: Normal

EEG: Generalised epileptiform discharges with left sided emphasis on a slow theta background, the epileptiform discharges activate only slightly in sleep. EEG felt to be more consistent with ASD than with EASD.

Genetic findings for Case 81

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
RBM15 (*de novo*) c.1177delG; p.A393fs*11	Low / Moderate to high	Encodes RNA-binding motif protein 15, a key regulator of RNA methylation, with important roles in many cellular processes including haematopoietic cell homeostasis.[1] It has also been implicated in the processes of neurulation and neural morphogenesis in early brain development.[2] The Drosophila ortholog *NITO,* has been implicated in axon outgrowth and synaptic plasticity in mature neurons.[3]	• NIL neurological	• A de novo frameshift mutation that is absent from all normal population databases • Classified as likely pathogenic on ACMG criteria • Possible role in neurodevelopment although not well established, and this proband has more of a neurodevelopmental phenotype than an epilepsy phenotype	• Not previously described in LKS/ • Only 1 case in this cohort and this proband does not have typical LKS.
LAMA5 (UF) c.4268C>T p.A1423V	Moderate /Moderate	Encodes laminin alpha-5, an extracellular matrix glycoprotein with a role in the regulation of dendritic spine density and synaptic stability in the hippocampus[4]	• SLI, DD, ASD, Sz (MA)[5]	• Gene function linked with LTP and Sz mechanisms • Some overlap in PDP and LKS – SLI, ASD, Sz • Variants in this gene identified in 3 families in this cohort	• Variant in this gene previously reported in only 1 child with SLI, DD, ASD, Sz. Not previously reported in LKS • Some variants in this cohort were inherited from unaffected parents, no data on IP available.

[1](Hiriart et al., 2005); [2](Xie et al., 2019); [3](Gu et al., 2017) [4](Omar et al., 2017); [5](Han et al., 2018)

Case 84 (Atypical- global regression of skills)

Case summary:

Case 84 is a girl who presented to GOSH DEC at 8 years 11 months of age. Her parents did not have concerns until she was 6 years old, when they noted global regression of skills. She became unsteady and clumsy on her feet and had difficulty climbing stairs. She started having difficulty feeding herself, and her handwriting deteriorated. She also started having difficulty responding to questions and partaking in conversation. She was extremely irritable and emotional, and her vision deteriorated to the point of her needing a magnifying glass at school. Clinical seizures manifest at 8 years of age, these included right focal motor seizures, myoclonic seizures, absences and drop attacks. EEG at this age showed ESES. Neurometabolic testing was non-conclusive. Low galactocerebrosidase levels were detected on 2 occasions. This was accounted for by a predicted pseudo-deficiency allele on specific *GALC* testing, which excluded a diagnosis of Krabbe disease. Her vision stabilized as she got older. No definitive cause was found for her visual deterioration. Her VEP and ERG were normal. She achieved some partial seizure control on Levetiracetam with plateauing of her developmental skills. Steroid therapy at 9 years 4 months of age afforded some benefit – there was some reduction in seizure burden and she became more alert and talkative. At her last formal assessment at 9 years 7 months of age, she continued to have severe impairment in both verbal and non-verbal skills.

Early development: Speech and language delay

Co-morbidities: Behavioural difficulties- mood swings and tantrums, ID, motor coordination difficulties

Family history: Parents are first cousins. Mother's brother had febrile seizures.

Dysmorphic features: NIL

Examination findings: Normal neurological examination. Weight: 50^{th}-75^{th} centile, height: 25^{th} centile, head circumference: 0.4^{th}-2^{nd} centile.

MRI: Non-progressive, non-specific white matter lesions – otherwise no localizing or lateralizing features

EEG: Multi-focal spike wave complexes with bilateral bursts. Spike discharges are significantly activated in sleep and become continuous, meeting criteria for ESES.

Genetic findings for Case 84

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
SLC9A9 (UM) c.481T>A p.S161T	Moderate	Solute Carrier 9A, member 9 – encodes Na+/H+ Exchanger 9, a protein that is located on the membranes of late recycling endosomes. This protein has been implicated in LTP as its role includes the recycling and degradation of neurotransmitter receptors and transporters.[1]	ASD with Sz (MA/BA)[2] ADHD (MA)[3] EASD (CNV, MA)[4]	Function of gene suggests possible link to LTP/Sz Significant overlap in clinical features between LKS and previously described phenotypes	Inherited from UM, there is a positive FH on the maternal side but there is no data available on IP Only 1 case identified in this cohort Variant has been classified as a VUS
RBFOX1 (UM) c.514C>T p.R172W	Moderate	Encodes RNA- binding FOX1 Homolog 1, an RNA binding protein that regulates alternative splicing of neuronal transcripts. This protein has a role in regulating LTP as it regulates the expression of an isoform of brain derived neurotrophic factor (BDNF) tyroskine kinase receptor.[4] RBFOX1 also has important roles in cortical development, neuronal maturation inhibitory synaptic transmission and excitatory synapse downscaling.[5-8]	ASD (MA)[9] GE/FE (CNV, MA)[10] TLE (MA)[11] CECTS (MA)[12]	Function of gene links strongly to LTP/Sz mechanisms Significant overlap in clinical features between LKS and previously described phenotypes especially CECTS, FE with SLI	Inherited from UM, there is a positive FH on the maternal side but there is no data available on IP Only 1 case identified in this cohort Variant has been classified as a VUS

[1](Zhang-James et al., 2019); [2](Morrow et al., 2008); [3](Lasky-Su et al., 2008); [4](Reinthaler et al., 2014); [5](Wamsley et al., 2018); [6](Jacko et al., 2018); [7](Vuong et al., 2018); [8](Rajman et al., 2017) [9](Bacchelli et al., 2020); [10](Pérez-Palma et al., 2017), [11](Krenn et al., 2020); [12](Lal et al., 2013);

Case 86 (Atypical – no clear regression)

Case summary:

A girl who presented at the age of 8 years with a long history of speech and language difficulties and verbal dyspraxia with slow progress despite regular speech and language therapy. There was no history of speech and language regression and no history of clinical seizures. EEG showed focal discharges which activated in sleep but did not meet criteria for ESES. She was treated with Sulthiame with some subjective benefit reported by her parents and reduction of focal discharges on EEG, but no objective improvement in speech and language assessment or academic performance. Sulthiame was eventually stopped with no sign of clinical deterioration. Treatment with steroids was discussed but eventually the team and parents decided against this. Her management currently consists of mainly learning and behavioural support.

Early development: Speech and language difficulties. Babbled at 18 months but only single words at 3 years with slow progress. Cruised at 1 year and walked at 22 months. Other development within normal limits.

Co-morbidities: Autism spectrum disorder, dyslexia, low average cognition

Family history: NIL

Dysmorphic features: NIL

Examination findings: Normal neurological examination.

MRI: Normal

EEG: Frequent bifrontal epileptiform discharges maximal anteriorly that activate in sleep up to about 50% of sleep recording.

Genetic findings for Case 86

Gene (Inheritance)/ Variant:	Significance for LKS/Proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
SETD5 (UF) c.2462G>A; p.C821Y	Moderate/ Moderate	Encodes a protein with histone methyltransferase activity and regulates neural stem cell proliferation, synapse formation and glutamatergic transmission[1]	• WS (MA)[2] • ASD, ID, facial dysmorphism (MA)[3,4]	• Gene function linked with Sz and LTP mechaniisms • Overlap in PDP and LKS: Sz, DD, ASD, MD • 2 cases in this cohort	• UF. However, IP has been reported. • VUS
FOXP2 (APS) c.1514C p.S505*	Moderate/ High *top candidate gene for this proband	Encodes Foxhead box P protein 2, a member of a family of transcription factors. *FOXP2* is thought to coordinate pathways important for brain development and function, including synaptogenesis[5,6].	• NDD with SLI, ASD, ID (MA)[5] • ECSWS (MA)[7]	• Gene function links to LTP and SLI mechanisms • Overlap in PDP and LKS: SLI, ASD, Sz • PTV in a gene where LOF is pathogenic[8] • Classified as pathogenic • Identified in other cohorts*	• 1 case is UM. However IP has previously been well described for this gene in SLI[8]
DIP2B (APS) c.295C>G p.R99G	Moderate/ Moderate	Encodes disco interacting protein 2, with important roles in dendritic outgrowth and synaptic transmission in the hippocampus.[9]	• NDD with ID +/- Sz (CGG repeat expansion)[10] • LKS (CNV, MA)[11]	• Gene function links to Sz and LTP mechanisms • CNV previously reported in 1 individual with LKS	• Classified as VUS • No other cases in this LKS cohort
LAMA5 (APS) c.4906C>T p.R1636C	Moderate/ Moderate	Encodes laminin alpha-5, an extracellular matrix glycoprotein with a role in the regulation of dendritic spine density and synaptic stability in the hippocampus[12]	• 1 child with SLI, DD, ASD, Sz (MA)[13]	• Gene function links to LTP and Sz mechanisms • Overlap in PDP and LKS: SLI, ASD, Sz • Variants identified in 3 families in this cohort	• Not previously reported in LKS • Some variants in this cohort were inherited from unaffected parents, no data on IP available.
CSPP1 (APS) c.1070C>A p.S357Y	Low/low	Encodes a core centrosomal protein that has a role in ciliary axoneme formation and cell-differentiation.[15]	• Joubert Syndrome (JS) (BA)[16] • CECTS (CNV, MA)[17]	• Phenotype overlap in CECTS and LKS • Variants identified in 2 families in this cohort	• The other variant was UF, but there is IP in *CSPP1* ciliopathies[18] • CNV in CECTS included *ARFGEF1*, a better DEE candidate gene. [17]

[1](Sessa et al., 2019); [2](Kobayashi et al., 2016); [3](Fernandes et al., 2018); [4](Powis et al., 2018); [5](Co et al., 2020); [6](Sia et al., 2013); [7](Lesca et al., 2012); [8](Estruch et al., 2016); [9](Xing et al., 2020); [10](Winnepenninckx et al., 2007); [11](Conroy et al., 2014); [12](Omar et al., 2017); [13](Han et al., 2018); [15](Frikstad et al., 2019); [16](Shaheen et al., 2014) [17](Addis et al., 2018); [18](Ben-Omran et al., 2015) *2 cases within this LKS cohort (3.7%) and 2 cases within Lesca et al's similar cohort of LKS/ECSWS patients (3.2%)

Case 90 (Atypical)

Case summary:

Case 90 presented at the age of 4 years with a history of GTCS arising from sleep. There was also a history of drop attacks which responded to prednisolone therapy in Portugal. His mother did not have any worries about his development until he entered year one of school, when it became apparent, he had difficulties with attention and was struggling to draw pictures and write letters. There was no clear history of regression. Speech and language assessments demonstrated severe language disorder and moderate ID. His seizures responded to treatment with AEDs and steroids, and there was some reported improvement in his cognitive abilities, but he continued to have severe language impairment.

Early development: Within normal limits – walked at 14 months, 1st words by 2 years.

Co-morbidities: ADHD, moderate ID

Family history: His father has a history of epilepsy. Paternal DNA was unavailable due to parental separation. His mother has a history of ADHD.

Dysmorphic features: NIL

Examination findings: Normal

MRI: Normal at 5 years

EEG: occasional discharges seen over the left centroparietal region, in sleep the discharges become more frequent and widespread, meeting criteria for ESES.

Genetic findings for Case 90

Gene (Inheritance)/ Variant:	Significance to LKS/proband	Function:	Disorders associated with:	Considerations- causative/contributary significance for LKS/proband	
				For:	Against:
GRIN2D (UM) c.1244T>C; p.L415P	Moderate/ moderate	Encodes N2D subunit of NMDA receptor a receptor with a role in excitatory neurotransmission and LTP[1].	Epilepsy with ID, most with severe developmental delay (MA)[1] Schizophrenia (MA)[1] ASD (MA)[1]	Gene function can be linked with seizure manifestation and LTP processes. Previously described in other DEE with some overlap in phenotype with LKS -Sz, ID, DD	Inherited from mother, who only has ADHD -no available data on IP Only 1 case in this LKS cohort, not previously described in LKS VUS
MBD5 (UM) c.599G>A p.R200Q	Moderate/ Moderate to high	Encodes methyl-CpG-binding domain (MBD) protein 5. Like other MBD proteins, MBD5, has a role in transcriptional activation. It has also been found to have a role in neurite outgrowth and neuronal differentiation.[2,3]	MBD5-associated neurodevelopmental disorder[3] Sz of various semiology. ID, BD, ASD, ADHD, SLI, MD, FTT, dysmorphic features	Function of gene suggests possible link with LTP, Sz Some overlap of clinical features for previously described phenotypes and this proband/LKS – ID, ASD, SLI Same variant submitted on ClinVar albeit as VUS, with similar phenotypes – ID, DD, with Sz	Inherited from mother who only has ADHD, but IP has been reported[3]. Only 1 case in this cohort VUS
LTBP1 (UM) c.4217G>A p.G1406D	Moderate	Encodes latent transforming growth factor β binding protein-1 a component of the extracellular matrix, which regulates the function of latent TGF- β.[4,5] The function of this protein within the CNS is not clear. A study looking at the transcriptomic profiles of a murine model of TLE has identified *LTBP1* as a key regulator in epileptogenesis. [5]	EASD (CNV, MA)[6]	Function of gene may have link to epileptogenesis Part of a CNV identified in a patient with EASD[6] Variants in this gene were identified in 2 individuals within this LKS cohort (also found in Case 74)	Although previously described in an individual with EASD this was as part of a CNV, encompassing several other genes.[6] Inherited from mother who only has ADHD, no data on IP is available. Both variants have been classified as VUS

[1](Camp and Yuan, 2020); [2](Camarena et al., 2014); [3](Mullegama and Elsea, 2016); [4](Robertson et al., 2015); [5](Fu et al., 2020) ; [6](Reinthaler et al., 2014)

Abbreviations

a.a: amino acid; abN: abnormality; AD: Alzheimer's disease; ADHD: attention deficit hyperactivity disorder; AED: anti-epileptic drug; AIS: auto-inflammatory syndromes; aka: also known as; APS: Awaiting parental sequencing; AM: affected mother; AUM: Absent in unaffected mother, ASD: autistic spectrum disorder, ATS: atonic seizures; a/w: associated with

BA: biallelic; BBB: blood-brain barrier; BD: behavioural difficulties; BFNS: benign familial neonatal epilepsy, BFIS: Benign familial infantile epilepsy; BP: biparental

CECTS: childhood epilepsy with centrotemporal spikes; CNS: central nervous system; CNV: copy number variant; CP: cerebral palsy; CTS: centrotemporal spikes;

DD: developmental delay; DEC: developmental epilepsy clinic; DEE: developmental epileptic encephalopathy; DNA: deoxyribonucleic acid; DNV: de novo variant; DS: Dravet Syndrome; DysF: dysmorphic features;

EASD: epilepsy aphasia spectrum disorders; ECSWS: epilepsy with continuous spike waves in slow wave sleep; EEG: electroencephalogram; EIMFS: Epilepsy of infancy with migrating focal seizures; EOAE: early onset absence epilepsy; ESES: electrical status epilepticus in slow wave sleep; ERG: electroretinogram

FE: focal epilepsy; FFEVF: Familial focal epilepsy with varying foci; FS: frameshift; FSz: focal seizures

GABA: gamma-amino butyric acid; GDD: global developmental delay; GE: generalised epilepsy; GMH: grey matter heterotopia; GOSH: Great Ormond Street Hospital

ID: intellectual disability; IP: Incomplete penetrance

JME: juvenile myoclonic epilepsy;

LGS: Lennox-Gastaut syndrome; LKS: Landau Kleffner Syndrome; LTP: long term potentiation;

MA: monoallelic; MaC: macrocephaly; MD: movement disorder; MiC: microcephaly; MRI:magnetic resonance imaging; MS: multiple sclerosis;

NDD: neurodevelopmental disorder; NPLHS: neurodevelopmental disorder with poor language and loss of hand skills; NPS; no parental samples

PDB: population databases; PDP: Previously described phenotypes; PECS: picture exchange communication system; PTBP: predicted to be pathogenic; PTV: protein truncating variant;

SkAbn: skeletal abnormalities; SLI: speech and language impairment; SWI: spike wave index; Sz: seizures;

TLE: temporal lobe epilepsy

UF: Inherited from unaffected father; UM: inherited from unaffected mother;

VEP: visual evoked potential; VUS: variant of uncertain significance;

WM: white matter; WS: West Syndrome

9 References

ABDEL-SALAM, G., THOENES, M., AFIFI, H. H., KORBER, F., SWAN, D. & BOLZ, H. J. 2014. The supposed tumor suppressor gene WWOX is mutated in an early lethal microcephaly syndrome with epilepsy, growth retardation and retinal degeneration. *Orphanet J Rare Dis,* 9**,** 12.

ABDELHADI, O., IANCU, D., STANESCU, H., KLETA, R. & BOCKENHAUER, D. 2016. EAST syndrome: Clinical, pathophysiological, and genetic aspects of mutations in KCNJ10. *Rare Dis,* 4**,** e1195043.

ABE, K., CHISAKA, O., VAN ROY, F. & TAKEICHI, M. 2004. Stability of dendritic spines and synaptic contacts is controlled by alpha N-catenin. *Nat Neurosci,* 7**,** 357-63.

ABRAHAM, W. C. & WILLIAMS, J. M. 2003. Properties and mechanisms of LTP maintenance. *Neuroscientist,* 9**,** 463-74.

ADDIS, L., SPROVIERO, W., THOMAS, S. V., CARABALLO, R. H., NEWHOUSE, S. J., GOMEZ, K., HUGHES, E., KINALI, M., MCCORMICK, D., HANNAN, S., COSSU, S., TAYLOR, J., AKMAN, C. I., WOLF, S. M., MANDELBAUM, D. E., GUPTA, R., VAN DER SPEK, R. A., PRUNA, D. & PAL, D. K. 2018. Identification of new risk factors for rolandic epilepsy: CNV at Xp22.31 and alterations at cholinergic synapses. *J Med Genet*.

ADDIS, L., VIRDEE, J. K., VIDLER, L. R. & COLLIER, D. A. 2017. Epilepsy-associated GRIN2A mutations reduce NMDA receptor trafficking and agonist potency - molecular profiling and functional rescue. 7**,** 66.

ADZHUBEI, I., JORDAN, D. M. & SUNYAEV, S. R. 2013. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet,* Chapter 7**,** Unit7.20.

AEPPLI, T. R., RYMEN, D., ALLEGRI, G., BODE, P. K. & HÄBERLE, J. 2020. Glycogen storage disease type VI: clinical course and molecular background. *Eur J Pediatr,* 179**,** 405-413.

AIKAWA, T., HOLM, M. L. & KANEKIYO, T. 2018. ABCA7 and Pathogenic Pathways of Alzheimer's Disease. *Brain Sci,* 8.

AIT-EL-MKADEM, S., DAYEM-QUERE, M., GUSIC, M., CHAUSSENOT, A., BANNWARTH, S., FRANCOIS, B., GENIN, E. C., FRAGAKI, K., VOLKER-TOUW, C. L. M., VASNIER, C., SERRE, V., VAN GASSEN, K. L. I., LESPINASSE, F., RICHTER, S., EISENHOFER, G., ROUZIER, C., MOCHEL, F., DE SAINT-MARTIN, A., ABI WARDE, M. T., DE SAIN-VAN DER VELDE, M. G. M., JANS, J. J. M., AMIEL, J., AVSEC, Z., MERTES, C., HAACK, T. B., STROM, T., MEITINGER, T., BONNEN, P. E., TAYLOR, R. W., GAGNEUR, J., VAN HASSELT, P. M., ROTIG, A., DELAHODDE, A., PROKISCH, H., FUCHS, S. A. & PAQUIS-FLUCKLINGER, V. 2017. Mutations in MDH2, Encoding a Krebs Cycle Enzyme, Cause Early-Onset Severe Encephalopathy. *Am J Hum Genet,* 100**,** 151-159.

AL-MEHMADI, S., SPLITT, M., RAMESH, V., DEBROSSE, S., DESSOFFY, K., XIA, F., YANG, Y., ROSENFELD, J. A., COSSETTE, P., MICHAUD, J. L., HAMDAN, F. F., CAMPEAU, P. M. & MINASSIAN, B. A. 2016. FHF1 (FGF12) epileptic encephalopathy. *Neurol Genet,* 2**,** e115.

ALAZAMI, A. M., HIJAZI, H., KENTAB, A. Y. & ALKURAYA, F. S. 2014. NECAP1 loss of function leads to a severe infantile epileptic encephalopathy. *J Med Genet,* 51**,** 224-8.

ALAZAMI, A. M., PATEL, N., SHAMSELDIN, H. E., ANAZI, S., AL-DOSARI, M. S., ALZAHRANI, F., HIJAZI, H., ALSHAMMARI, M., ALDAHMESH, M. A., SALIH, M. A., FAQEIH, E., ALHASHEM, A., BASHIRI, F. A., AL-OWAIN, M., KENTAB, A. Y., SOGATY, S., AL TALA, S., TEMSAH, M. H., TULBAH, M., ALJELAIFY, R. F., ALSHAHWAN, S. A., SEIDAHMED, M. Z., ALHADID, A. A., ALDHALAAN, H., ALQALLAF, F., KURDI, W., ALFADHEL, M., BABAY, Z., ALSOGHEER, M., KAYA, N., AL-HASSNAN, Z. N., ABDEL-SALAM, G. M., AL-SANNAA, N., AL MUTAIRI, F., EL KHASHAB, H. Y., BOHLEGA, S., JIA, X., NGUYEN, H. C., HAMMAMI, R., ADLY, N., MOHAMED, J. Y., ABDULWAHAB, F., IBRAHIM, N., NAIM, E. A., AL-YOUNES, B., MEYER, B. F., HASHEM, M., SHAHEEN, R., XIONG, Y., ABOUELHODA, M., ALDEERI, A. A., MONIES, D. M. & ALKURAYA, F. S. 2015. Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. *Cell Rep,* 10**,** 148-61.

ALFAIZ, A. A., MULLER, V., BOUTRY-KRYZA, N., VILLE, D., GUEX, N., DE BELLESCIZE, J., RIVIER, C., LABALME, A., DES PORTES, V., EDERY, P., TILL, M., XENARIOS, I., SANLAVILLE, D., HERRMANN, J. M., LESCA, G. & REYMOND, A. 2016. West syndrome caused by homozygous variant in the evolutionary conserved gene encoding the mitochondrial elongation factor GUF1. *Eur J Hum Genet,* 24**,** 1001-8.

ALTROCK, W. D., TOM DIECK, S., SOKOLOV, M., MEYER, A. C., SIGLER, A., BRAKEBUSCH, C., FASSLER, R., RICHTER, K., BOECKERS, T. M., POTSCHKA, H., BRANDT, C., LOSCHER, W., GRIMBERG, D., DRESBACH, T., HEMPELMANN, A., HASSAN, H., BALSCHUN, D., FREY, J. U., BRANDSTATTER, J. H., GARNER, C. C., ROSENMUND, C. & GUNDELFINGER, E. D. 2003. Functional inactivation of a fraction of excitatory synapses in mice deficient for the active zone protein bassoon. *Neuron,* 37**,** 787-800.

ALTSHULER, D., DALY, M. J. & LANDER, E. S. 2008. Genetic mapping in human disease. *Science,* 322**,** 881-8.

ANAZI, S., MADDIREVULA, S., FAQEIH, E., ALSEDAIRY, H., ALZAHRANI, F., SHAMSELDIN, H. E., PATEL, N., HASHEM, M., IBRAHIM, N., ABDULWAHAB, F., EWIDA, N., ALSAIF, H. S., AL SHARIF, H., ALAMOUDI, W., KENTAB, A., BASHIRI, F. A., ALNASER, M., ALWADEI, A. H., ALFADHEL, M., EYAID, W., HASHEM, A., AL ASMARI, A., SALEH, M. M., ALSAMAN, A., ALHASAN, K. A., ALSUGHAYIR, M., AL SHAMMARI, M., MAHMOUD, A., AL-HASSNAN, Z. N., AL-HUSAIN, M., OSAMA KHALIL, R., ABD EL MEGUID, N., MASRI, A., ALI, R., BEN-OMRAN, T., EL FISHWAY, P., HASHISH, A., ERCAN SENCICEK, A., STATE, M., ALAZAMI, A. M., SALIH, M. A., ALTASSAN, N., AROLD, S. T., ABOUELHODA, M., WAKIL, S. M., MONIES, D., SHAHEEN, R. & ALKURAYA, F. S. 2017. Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. *Mol Psychiatry,* 22**,** 615-624.

ANDERSON, M. P. 2018. DEPDC5 takes a second hit in familial focal epilepsy. *J Clin Invest,* 128**,** 2194-2196.

ANDERSON, V., JACOBS, R., SPENCER-SMITH, M., COLEMAN, L., ANDERSON, P., WILLIAMS, J., GREENHAM, M. & LEVENTER, R. 2010. Does early age at brain insult predict worse outcome? Neuropsychological implications. *J Pediatr Psychol,* 35**,** 716-27.

ARNADOTTIR, G. A., JENSSON, B. O., MARELSSON, S. E., SULEM, G., ODDSSON, A., KRISTJANSSON, R. P., BENONISDOTTIR, S., GUDJONSSON, S. A., MASSON, G., THORISSON, G. A., SAEMUNDSDOTTIR, J., MAGNUSSON, O. T., JONASDOTTIR, A., JONASDOTTIR, A., SIGURDSSON, A., GUDBJARTSSON, D. F., THORSTEINSDOTTIR, U., ARNGRIMSSON, R. & SULEM, P. 2017. Compound heterozygous mutations in UBA5 causing early-onset epileptic encephalopathy in two sisters. 18**,** 103.

ASSENZA, G., BENVENGA, A., GENNARO, E., TOMBINI, M., CAMPANA, C., ASSENZA, F., DI PINO, G. & DI LAZZARO, V. 2017. A novel c132-134del mutation in Unverricht-Lundborg disease and the review of literature of heterozygous compound patients. *Epilepsia,* 58**,** e31-e35.

ASSOUM, M., PHILIPPE, C., ISIDOR, B., PERRIN, L., MAKRYTHANASIS, P., SONDHEIMER, N., PARIS, C., DOUGLAS, J., LESCA, G., ANTONARAKIS, S., HAMAMY, H., JOUAN, T., DUFFOURD, Y., AUVIN, S., SAUNIER, A., BEGTRUP, A., NOWAK, C., CHATRON, N., VILLE, D., MIRESKANDARI, K., MILANI, P., JONVEAUX, P., LEMEUR, G., MILH, M., AMAMOTO, M., KATO, M., NAKASHIMA, M., MIYAKE, N., MATSUMOTO, N., MASRI, A., THAUVIN-ROBINET, C., RIVIERE, J. B., FAIVRE, L. & THEVENON, J. 2016. Autosomal-Recessive Mutations in AP3B2, Adaptor-Related Protein Complex 3 Beta 2 Subunit, Cause an Early-Onset Epileptic Encephalopathy with Optic Atrophy. *Am J Hum Genet,* 99**,** 1368-1376.

BACCHELLI, E., CAMELI, C., VIGGIANO, M., IGLIOZZI, R., MANCINI, A., TANCREDI, R., BATTAGLIA, A. & MAESTRINI, E. 2020. An integrated analysis of rare CNV and exome variation in Autism Spectrum Disorder using the Infinium PsychArray. *Sci Rep,* 10**,** 3198.

BACCHELLI, E., CERONI, F., PINTO, D., LOMARTIRE, S., GIANNANDREA, M., D'ADAMO, P., BONORA, E., PARCHI, P., TANCREDI, R., BATTAGLIA, A. & MAESTRINI, E. 2014. A CTNNA3 compound heterozygous deletion implicates a role for αT-catenin in susceptibility to autism spectrum disorder. *J Neurodev Disord,* 6**,** 17.

BAGASRAWALA, I., MEMI, F., N, V. R. & ZECEVIC, N. 2017. N-Methyl d-Aspartate Receptor Expression Patterns in the Human Fetal Cerebral Cortex. *Cereb Cortex,* 27**,** 5041-5053.

BAHI-BUISSON, N., KAMINSKA, A., BODDAERT, N., RIO, M., AFENJAR, A., GERARD, M., GIULIANO, F., MOTTE, J., HERON, D., MOREL, M. A., PLOUIN, P., RICHELME, C., DES PORTES, V., DULAC, O., PHILIPPE, C., CHIRON, C., NABBOUT, R. & BIENVENU, T. 2008. The three stages of epilepsy in patients with CDKL5 mutations. *Epilepsia,* 49**,** 1027-37.

BAI, S. W., HERRERA-ABREU, M. T., ROHN, J. L., RACINE, V., TAJADURA, V., SURYAVANSHI, N., BECHTEL, S., WIEMANN, S., BAUM, B. & RIDLEY, A. J. 2011. Identification and characterization of a set of conserved and new regulators of cytoskeletal organization, cell morphology and migration. *BMC Biol,* 9**,** 54.

BALDASSARI, S., LICCHETTA, L., TINUPER, P., BISULLI, F. & PIPPUCCI, T. 2016. GATOR1 complex: the common genetic actor in focal epilepsies. *J Med Genet,* 53**,** 503-10.

BALDASSARI, S., PICARD, F., VERBEEK, N. E., VAN KEMPEN, M., BRILSTRA, E. H., LESCA, G., CONTI, V., GUERRINI, R., BISULLI, F., LICCHETTA, L., PIPPUCCI, T., TINUPER, P., HIRSCH, E., DE SAINT MARTIN, A., CHELLY, J., RUDOLF, G., CHIPAUX, M., FERRAND-SORBETS, S., DORFMÜLLER, G., SISODIYA, S., BALESTRINI, S., SCHOELER, N., HERNANDEZ-HERNANDEZ, L., KRITHIKA, S., OEGEMA, R., HAGEBEUK, E., GUNNING, B., DECKERS, C., BERGHUIS, B., WEGNER, I., NIKS, E., JANSEN, F. E., BRAUN, K., DE JONG, D., RUBBOLI, G., TALVIK, I., SANDER, V., ULDALL, P., JACQUEMONT, M. L., NAVA, C., LEGUERN, E., JULIA, S., GAMBARDELLA, A., D'ORSI, G., CRICHIUTTI, G., FAIVRE, L., DARMENCY, V., BENOVA, B., KRSEK, P., BIRABEN, A., LEBRE, A. S., JENNESSON, M., SATTAR, S., MARCHAL, C., NORDLI, D. R., JR., LINDSTROM, K., STRIANO, P., LOMAX, L. B., KISS, C., BARTOLOMEI, F., LEPINE, A. F., SCHOONJANS, A. S., STOUFFS, K., JANSEN, A., PANAGIOTAKAKI, E., RICARD-MOUSNIER, B., THEVENON, J., DE BELLESCIZE, J., CATENOIX, H., DORN, T., ZENKER, M., MÜLLER-SCHLÜTER, K., BRANDT, C., KREY, I., POLSTER, T., WOLFF, M., BALCI, M., ROSTASY, K., ACHAZ, G., ZACHER, P., BECHER, T., CLOPPENBORG, T., YUSKAITIS, C. J., WECKHUYSEN, S., PODURI, A., LEMKE, J. R., MØLLER, R. S. & BAULAC, S. 2019. The landscape of epilepsy-related GATOR1 variants. *Genet Med,* 21**,** 398-408.

BALESTRINI, S., MILH, M., CASTIGLIONI, C., LUTHY, K., FINELLI, M. J., VERSTREKEN, P., CARDON, A., STRAZISAR, B. G., HOLDER, J. L., JR., LESCA, G., MANCARDI, M. M., POULAT, A. L., REPETTO, G. M., BANKA, S., BILO, L., BIRKELAND, L. E., BOSCH, F., BROCKMANN, K., CROSS, J. H., DOUMMAR, D., FELIX, T. M., GIULIANO, F., HORI, M., HUNING, I., KAYSERILI, H., KINI, U., LEES, M. M., MEENAKSHI, G., MEWASINGH, L., PAGNAMENTA, A. T., PELUSO, S., MEY, A., RICE, G. M., ROSENFELD, J. A., TAYLOR, J. C., TROESTER, M. M., STANLEY, C. M., VILLE, D., WALKIEWICZ, M., FALACE, A., FASSIO, A., LEMKE, J. R., BISKUP, S., TARDIF, J., AJEAWUNG, N. F., TOLUN, A., CORBETT, M., GECZ, J., AFAWI, Z., HOWELL, K. B., OLIVER, K. L., BERKOVIC, S. F., SCHEFFER, I. E., DE FALCO, F. A., OLIVER, P. L., STRIANO, P., ZARA, F., CAMPEAU, P. M. & SISODIYA, S. M. 2016. TBC1D24 genotype-phenotype correlation: Epilepsies and other neurologic features. *Neurology,* 87**,** 77-85.

BALREIRA, A., GASPAR, P., CAIOLA, D., CHAVES, J., BEIRAO, I., LIMA, J. L., AZEVEDO, J. E. & MIRANDA, M. C. 2008. A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome. *Hum Mol Genet,* 17**,** 2238-43.

BALSCHUN, D., WOLFER, D. P., BERTOCCHINI, F., BARONE, V., CONTI, A., ZUSCHRATTER, W., MISSIAEN, L., LIPP, H. P., FREY, J. U. & SORRENTINO, V. 1999. Deletion of the ryanodine receptor type 3 (RyR3) impairs forms of synaptic plasticity and spatial learning. *Embo j,* 18**,** 5264-73.

BALU, D. T. & COYLE, J. T. 2011. Glutamate receptor composition of the post-synaptic density is altered in genetic mouse models of NMDA receptor hypo- and hyperfunction. *Brain Res,* 1392**,** 1-7.

BALU, D. T., TAKAGI, S., PUHL, M. D., BENNEYWORTH, M. A. & COYLE, J. T. 2014. D-serine and serine racemase are localized to neurons in the adult mouse and human forebrain. *Cell Mol Neurobiol,* 34**,** 419-35.

BANNE, E., ATAWNEH, O., HENNEKE, M., BROCKMANN, K., GÄRTNER, J., ELPELEG, O. & EDVARDSON, S. 2013. West syndrome, microcephaly, grey matter heterotopia and hypoplasia of corpus callosum due to a novel ARFGEF2 mutation. *J Med Genet,* 50**,** 772-5.

BARCIA, G., FLEMING, M. R., DELIGNIERE, A., GAZULA, V. R., BROWN, M. R., LANGOUET, M., CHEN, H., KRONENGOLD, J., ABHYANKAR, A., CILIO, R., NITSCHKE, P., KAMINSKA, A., BODDAERT, N., CASANOVA, J. L., DESGUERRE, I., MUNNICH, A., DULAC, O., KACZMAREK, L. K., COLLEAUX, L. & NABBOUT, R. 2012. De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. *Nat Genet,* 44**,** 1255-9.

BASEL-VANAGAITE, L., HERSHKOVITZ, T., HEYMAN, E., RASPALL-CHAURE, M., KAKAR, N., SMIRIN-YOSEF, P., VILA-PUEYO, M., KORNREICH, L., THIELE, H., BODE, H., LAGOVSKY, I., DAHARY, D., HAVIV, A., HUBSHMAN, M. W., PASMANIK-CHOR, M., NURNBERG, P., GOTHELF, D., KUBISCH, C., SHOHAT, M., MACAYA, A. & BORCK, G. 2013. Biallelic SZT2 mutations cause infantile encephalopathy with epilepsy and dysmorphic corpus callosum. *Am J Hum Genet,* 93**,** 524-9.

BASSI, M. T., BALOTTIN, U., PANZERI, C., PICCINELLI, P., CASTALDO, P., BARRESE, V., SOLDOVIERI, M. V., MICELI, F., COLOMBO, M., BRESOLIN, N., BORGATTI, R. & TAGLIALATELA, M. 2005. Functional analysis of novel KCNQ2 and KCNQ3 gene variants found in a large pedigree with benign familial neonatal convulsions (BFNC). *Neurogenetics,* 6**,** 185-93.

BAUMER, F. M., MCNAMARA, N. A., FINE, A. L., PESTANA-KNIGHT, E., SHELLHAAS, R. A., HE, Z., ARNDT, D. H., GAILLARD, W. D., KELLEY, S. A., NAGAN, M., OSTENDORF, A. P., SINGHAL, N. S., SPELTZ, L. & CHAPMAN, K. E. 2021. Treatment Practices and Outcomes in Continuous Spike and Wave During Slow Wave Sleep (CSWS): A Multicenter Collaboration. *J Pediatr*.

BAYNES, K., KEGL, J. A., BRENTARI, D., KUSSMAUL, C. & POIZNER, H. 1998. Chronic auditory agnosia following Landau-Kleffner syndrome: a 23 year outcome study. *Brain Lang,* 63**,** 381-425.

BEARDEN, D., STRONG, A., EHNOT, J., DIGIOVINE, M., DLUGOS, D. & GOLDBERG, E. M. 2014. Targeted treatment of migrating partial seizures of infancy with quinidine. *Ann Neurol,* 76**,** 457-61.

BECCHETTI, A., ARACRI, P., MENEGHINI, S., BRUSCO, S. & AMADEO, A. 2015. The role of nicotinic acetylcholine receptors in autosomal dominant nocturnal frontal lobe epilepsy. *Front Physiol,* 6**,** 22.

BELET, S., FIEREMANS, N., YUAN, X., VAN ESCH, H., VERBEECK, J., YE, Z., CHENG, L., BRODSKY, B. R., HU, H., KALSCHEUER, V. M., BRODSKY, R. A. & FROYEN, G. 2014. Early frameshift mutation in PIGA identified in a large XLID family without neonatal lethality. *Hum Mutat,* 35**,** 350-5.

BEN-OMRAN, T., ALSULAIMAN, R., KAMEL, H., SHAHEEN, R. & ALKURAYA, F. S. 2015. Intrafamilial clinical heterogeneity of CSPP1-related ciliopathy. *Am J Med Genet A,* 167a**,** 2478-80.

BERG, A. T. 2011. Epilepsy, cognition, and behavior: The clinical picture. *Epilepsia,* 52 Suppl 1**,** 7-12.

BERG, A. T., BERKOVIC, S. F., BRODIE, M. J., BUCHHALTER, J., CROSS, J. H., VAN EMDE BOAS, W., ENGEL, J., FRENCH, J., GLAUSER, T. A., MATHERN, G. W., MOSHE, S. L., NORDLI, D., PLOUIN, P. & SCHEFFER, I. E. 2010. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia,* 51**,** 676-85.

BERG, J. M. & GESCHWIND, D. H. 2012. Autism genetics: searching for specificity and convergence. *Genome Biol,* 13**,** 247.

BERGQVIST, A. G., CHEE, C. M., LUTCHKA, L. M. & BROOKS-KAYAL, A. R. 1999. Treatment of acquired epileptic aphasia with the ketogenic diet. *J Child Neurol,* 14**,** 696-701.

BHARDWAJ, P., SHARMA, V. K., SHARMA, R. & GAUTAM, P. 2009. Acquired epileptic aphasia: Landau-Kleffner syndrome. *J Pediatr Neurosci,* 4**,** 52-3.

BIENVENU, T., DIEBOLD, B., CHELLY, J. & ISIDOR, B. 2013. Refining the phenotype associated with MEF2C point mutations. *Neurogenetics,* 14**,** 71-5.

BISHOP, D. V. 1985. Age of onset and outcome in 'acquired aphasia with convulsive disorder' (Landau-Kleffner syndrome). *Dev Med Child Neurol,* 27**,** 705-12.

BISSEN, D., FOSS, F. & ACKER-PALMER, A. 2019. AMPA receptors and their minions: auxiliary proteins in AMPA receptor trafficking. *Cell Mol Life Sci,* 76**,** 2133-2169.

BLANCHARD, M. G., WILLEMSEN, M. H., WALKER, J. B., DIB-HAJJ, S. D., WAXMAN, S. G., JONGMANS, M. C. J., KLEEFSTRA, T., VAN DE WARRENBURG, B. P., PRAAMSTRA, P., NICOLAI, J., YNTEMA, H. G., BINDELS, R. J. M., MEISLER, M. H. & KAMSTEEG, E. J. 2015. De novo gain-of-function and loss-of-function mutations of SCN8A in patients with intellectual disabilities and epilepsy. *J Med Genet,* 52**,** 330-7.

BLISS, T. V. & COOKE, S. F. 2011. Long-term potentiation and long-term depression: a clinical perspective. *Clinics (Sao Paulo),* 66 Suppl 1**,** 3-17.

BLISS, T. V. & LOMO, T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol,* 232**,** 331-56.

BLÜTHGEN, N., VAN BENTUM, M., MERZ, B., KUHL, D. & HERMEY, G. 2017. Profiling the MAPK/ERK dependent and independent activity regulated transcriptional programs in the murine hippocampus in vivo. *Sci Rep,* 7**,** 45101.

BO, T., JIANG, Y., CAO, H., WANG, J. & WU, X. 2003. [Long-term effects of recurrent seizures in neonatal rats on NMDA receptor expression in the brain]. *Beijing Da Xue Xue Bao Yi Xue Ban,* 35**,** 292-5.

BOILLOT, M., HUNEAU, C., MARSAN, E., LEHONGRE, K., NAVARRO, V., ISHIDA, S., DUFRESNOIS, B., OZKAYNAK, E., GARRIGUE, J., MILES, R., MARTIN, B., LEGUERN, E., ANDERSON, M. P. & BAULAC, S. 2014. Glutamatergic neuron-targeted loss of LGI1 epilepsy gene results in seizures. *Brain,* 137**,** 2984-96.

BÖLSTERLI, B. K., GARDELLA, E., PAVLIDIS, E., WEHRLE, F. M., TASSINARI, C. A., HUBER, R. & RUBBOLI, G. 2017. Remission of encephalopathy with status epilepticus (ESES) during sleep renormalizes regulation of slow wave sleep. *Epilepsia,* 58**,** 1892-1901.

BÖLSTERLI HEINZLE, B. K., FATTINGER, S., KURTH, S., LEBOURGEOIS, M. K., RINGLI, M., BAST, T., CRITELLI, H., SCHMITT, B. & HUBER, R. 2014. Spike wave location and density disturb sleep slow waves in patients with CSWS (continuous spike waves during sleep). *Epilepsia,* 55**,** 584-91.

BORGATTI, R., ZUCCA, C., CAVALLINI, A., FERRARIO, M., PANZERI, C., CASTALDO, P., SOLDOVIERI, M. V., BASCHIROTTO, C., BRESOLIN, N., DALLA BERNARDINA, B., TAGLIALATELA, M. & BASSI, M. T. 2004. A novel mutation in KCNQ2 associated with BFNC, drug resistant epilepsy, and mental retardation. *Neurology,* 63**,** 57-65.

BOSCOLO, S., BALDAS, V., GOBBI, G., GIORDANO, L., CIONI, G., NOT, T., VENTURA, A. & TONGIORGI, E. 2005. Anti-brain but not celiac disease antibodies in Landau-Kleffner syndrome and related epilepsies. *J Neuroimmunol,* 160**,** 228-32.

BOWER, R., TRITSCHLER, D., VANDERWAAL, K., PERRONE, C. A., MUELLER, J., FOX, L., SALE, W. S. & PORTER, M. E. 2013. The N-DRC forms a conserved biochemical complex that maintains outer doublet alignment and limits microtubule sliding in motile axonemes. *Mol Biol Cell,* 24**,** 1134-52.

BROKER-LAI, J., KOLLEWE, A., SCHINDELDECKER, B., POHLE, J., NGUYEN CHI, V., MATHAR, I., GUZMAN, R., SCHWARZ, Y., LAI, A., WEISSGERBER, P., SCHWEGLER, H., DIETRICH, A., BOTH, M. & SPRENGEL, R. 2017. Heteromeric channels formed by TRPC1, TRPC4 and TRPC5 define hippocampal synaptic transmission and working memory. 36**,** 2770-2789.

BRÖKER-LAI, J., KOLLEWE, A., SCHINDELDECKER, B., POHLE, J., NGUYEN CHI, V., MATHAR, I., GUZMAN, R., SCHWARZ, Y., LAI, A., WEISSGERBE, P., SCHWEGLER, H., DIETRICH, A., BOTH, M., SPRENGEL, R., DRAGUHN, A., KÖHR, G., FAKLER, B., FLOCKERZI, V., BRUNS, D. & FREICHEL, M. 2017. Heteromeric channels formed by TRPC1, TRPC4 and TRPC5 define hippocampal synaptic transmission and working memory. *Embo j,* 36**,** 2770-2789.

BROWN, T. & LALOR, A. 2009. The Movement Assessment Battery for Children--Second Edition (MABC-2): a review and critique. *Phys Occup Ther Pediatr,* 29**,** 86-103.

BRUINSMA, C. F., SAVELBERG, S. M., KOOL, M. J., JOLFAEI, M. A., VAN WOERDEN, G. M., BAARENDS, W. M. & ELGERSMA, Y. 2016. An essential role for UBE2A/HR6A in learning and memory and mGLUR-dependent long-term depression. *Hum Mol Genet,* 25**,** 1-8.

BRUNKLAUS, A., ELLIS, R., REAVEY, E., SEMSARIAN, C. & ZUBERI, S. M. 2014. Genotype phenotype associations across the voltage-gated sodium channel family. *J Med Genet,* 51**,** 650-8.

BURGESS, R., WANG, S., MCTAGUE, A., BOYSEN, K. E., YANG, X., ZENG, Q., MYERS, K. A., ROCHTUS, A., TRIVISANO, M., GILL, D., SADLEIR, L. G., SPECCHIO, N., GUERRINI, R., MARINI, C., ZHANG, Y. H., MEFFORD, H. C., KURIAN, M. A., PODURI, A. H. & SCHEFFER, I. E. 2019. The Genetic Landscape of Epilepsy of Infancy with Migrating Focal Seizures. *Ann Neurol,* 86**,** 821-831.

BURMAKINA, S., GENG, Y., CHEN, Y. & FAN, Q. R. 2014. Heterodimeric coiled-coil interactions of human GABAB receptor. *Proc Natl Acad Sci U S A,* 111**,** 6958-63.

BUTLER, K. M., DA SILVA, C., SHAFIR, Y., WEISFELD-ADAMS, J. D., ALEXANDER, J. J., HEGDE, M. & ESCAYG, A. 2017. De novo and inherited SCN8A epilepsy mutations detected by gene panel analysis. *Epilepsy Res,* 129**,** 17-25.

BUTLER, M. G., RAFI, S. K., HOSSAIN, W., STEPHAN, D. A. & MANZARDO, A. M. 2015. Whole exome sequencing in females with autism implicates novel and candidate genes. *Int J Mol Sci,* 16**,** 1312-35.

BYUN, H., LEE, H. L., LIU, H., FORREST, D., RUDENKO, A. & KIM, I. J. 2019. Rorβ regulates selective axon-target innervation in the mammalian midbrain. *Development,* 146.

CAJAL 1911. *Histologie du système nerveux de l'homme & des vertébrés (translated)*, Oxford University Press.

CALLAERTS-VEGH, Z. A., T.; CLEMENTS, J.; ZWARTS, L.; ZUSCHRATTER, W.; BALSCHUN, D.; D'HOOGE, R.; CALLAERTS P 2009. Haploinsufficiency for PAX6 leads to impaired hippocampus dependent synaptic plasticity and behaviour. *Conference Abstract: 41st European Brain and Behaviour Society Meeting.* .

CAMARENA, V., CAO, L., ABAD, C., ABRAMS, A., TOLEDO, Y., ARAKI, K., ARAKI, M., WALZ, K. & YOUNG, J. I. 2014. Disruption of Mbd5 in mice causes neuronal functional deficits and neurobehavioral abnormalities consistent with 2q23.1 microdeletion syndrome. *EMBO Mol Med,* 6**,** 1003-15.

CAMP, C. R. & YUAN, H. 2020. GRIN2D/GluN2D NMDA receptor: Unique features and its contribution to pediatric developmental and epileptic encephalopathy. *Eur J Paediatr Neurol,* 24**,** 89-99.

CAPOVILLA, G., WOLF, P., BECCARIA, F. & AVANZINI, G. 2013. The history of the concept of epileptic encephalopathy. *Epilepsia,* 54 Suppl 8**,** 2-5.

CARABALLO, R. H., CEJAS, N., CHAMORRO, N., KALTENMEIER, M. C., FORTINI, S. & SOPRANO, A. M. 2014. Landau-Kleffner syndrome: a study of 29 patients. *Seizure,* 23**,** 98-104.

CARRANZA ROJO, D., HAMIWKA, L., MCMAHON, J. M., DIBBENS, L. M., ARSOV, T., SULS, A., STODBERG, T., KELLEY, K., WIRRELL, E., APPLETON, B., MACKAY, M., FREEMAN, J. L., YENDLE, S. C., BERKOVIC, S. F., BIENVENU, T., DE JONGHE, P., THORBURN, D. R., MULLEY, J. C., MEFFORD, H. C. & SCHEFFER, I. E. 2011. De novo SCN1A mutations in migrating partial seizures of infancy. *Neurology,* 77**,** 380-3.

CARVILL, G. L., HEAVIN, S. B., YENDLE, S. C., MCMAHON, J. M., O'ROAK, B. J., COOK, J., KHAN, A., DORSCHNER, M. O., WEAVER, M., CALVERT, S., MALONE, S., WALLACE, G., STANLEY, T., BYE, A. M., BLEASEL, A., HOWELL, K. B., KIVITY, S., MACKAY, M. T., RODRIGUEZ-CASERO, V., WEBSTER, R., KORCZYN, A., AFAWI, Z., ZELNICK, N., LERMAN-SAGIE, T., LEV, D., MOLLER, R. S., GILL, D., ANDRADE, D. M., FREEMAN, J. L., SADLEIR, L. G., SHENDURE, J., BERKOVIC, S. F., SCHEFFER, I. E. & MEFFORD, H. C. 2013a. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet,* 45**,** 825-30.

CARVILL, G. L., LIU, A., MANDELSTAM, S., SCHNEIDER, A., LACROIX, A., ZEMEL, M., MCMAHON, J. M., BELLO-ESPINOSA, L., MACKAY, M., WALLACE, G. & WAAK, M. 2018. Severe infantile onset developmental and epileptic encephalopathy caused by mutations in autophagy gene WDR45. 59**,** e5-e13.

CARVILL, G. L., MCMAHON, J. M., SCHNEIDER, A., ZEMEL, M., MYERS, C. T., SAYKALLY, J., NGUYEN, J., ROBBIANO, A., ZARA, F., SPECCHIO, N., MECARELLI, O., SMITH, R. L., LEVENTER, R. J., MOLLER, R. S., NIKANOROVA, M., DIMOVA, P., JORDANOVA, A., PETROU, S., HELBIG, I., STRIANO, P., WECKHUYSEN, S., BERKOVIC, S. F., SCHEFFER, I. E. & MEFFORD, H. C. 2015. Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures. *Am J Hum Genet,* 96**,** 808-15.

CARVILL, G. L., REGAN, B. M., YENDLE, S. C., O'ROAK, B. J., LOZOVAYA, N., BRUNEAU, N., BURNASHEV, N., KHAN, A., COOK, J., GERAGHTY, E., SADLEIR, L. G., TURNER, S. J., TSAI, M. H., WEBSTER, R., OUVRIER, R., DAMIANO, J. A., BERKOVIC, S. F., SHENDURE, J., HILDEBRAND, M. S., SZEPETOWSKI, P., SCHEFFER, I. E. & MEFFORD, H. C. 2013b. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. *Nat Genet,* 45**,** 1073-6.

CARVILL, G. L., WECKHUYSEN, S., MCMAHON, J. M., HARTMANN, C., MOLLER, R. S., HJALGRIM, H., COOK, J., GERAGHTY, E., O'ROAK, B. J., PETROU, S., CLARKE, A., GILL, D., SADLEIR, L. G., MUHLE, H., VON SPICZAK, S., NIKANOROVA, M., HODGSON, B. L., GAZINA, E. V., SULS, A., SHENDURE, J., DIBBENS, L. M., DE JONGHE, P., HELBIG, I., BERKOVIC, S. F., SCHEFFER, I. E. & MEFFORD, H. C. 2014. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. *Neurology,* 82**,** 1245-53.

CASSANDRI, M., SMIRNOV, A., NOVELLI, F., PITOLLI, C., AGOSTINI, M., MALEWICZ, M., MELINO, G. & RASCHELLÀ, G. 2017. Zinc-finger proteins in health and disease. *Cell Death Discov,* 3**,** 17071.

CATTERALL, W. A. 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron,* 26**,** 13-25.

CATTERALL, W. A., KALUME, F. & OAKLEY, J. C. 2010. NaV1.1 channels and epilepsy. *J Physiol,* 588**,** 1849-59.

CHAN, A. J. S., CYTRYNBAUM, C., HOANG, N., AMBROZEWICZ, P. M., WEKSBERG, R., DRMIC, I., RITZEMA, A., SCHACHAR, R., WALKER, S., UDDIN, M., ZARREI, M., YUEN, R. K. C. & SCHERER, S. W. 2019. Expanding the neurodevelopmental phenotypes of individuals with de novo KMT2A variants. *NPJ Genom Med,* 4**,** 9.

CHAN, E. M., YOUNG, E. J., IANZANO, L., MUNTEANU, I., ZHAO, X., CHRISTOPOULOS, C. C., AVANZINI, G., ELIA, M., ACKERLEY, C. A., JOVIC, N. J., BOHLEGA, S., ANDERMANN, E., ROULEAU, G. A., DELGADO-ESCUETA, A. V., MINASSIAN, B. A. & SCHERER, S. W. 2003. Mutations in NHLRC1 cause progressive myoclonus epilepsy. *Nat Genet,* 35**,** 125-7.

CHAPMAN, K. E., SPECCHIO, N., SHINNAR, S. & HOLMES, G. L. 2015. Seizing control of epileptic activity can improve outcome. *Epilepsia,* 56**,** 1482-5.

CHEN, J., ZHANG, J., LIU, A., ZHANG, L., LI, H., ZENG, Q., YANG, Z., YANG, X., WU, X. & ZHANG, Y. 2020a. CHD2-related epilepsy: novel mutations and new phenotypes. *Dev Med Child Neurol,* 62**,** 647-653.

CHEN, P., LI, Z., NIE, J., WANG, H., YU, B., WEN, Z., SUN, Y., SHI, X., JIN, L. & WANG, D. W. 2020b. MYH7B variants cause hypertrophic cardiomyopathy by activating the CaMK-signaling pathway. *Sci China Life Sci*.

CHEN, W., SHIEH, C., SWANGER, S. A., TANKOVIC, A., AU, M., MCGUIRE, M., TAGLIATI, M., GRAHAM, J. M., MADAN-KHETARPAL, S., TRAYNELIS, S. F., YUAN, H. & PIERSON, T. M. 2017a. GRIN1 mutation associated with intellectual disability alters NMDA receptor trafficking and function. *J Hum Genet,* 62**,** 589-597.

CHEN, W., TANKOVIC, A., BURGER, P. B., KUSUMOTO, H., TRAYNELIS, S. F. & YUAN, H. 2017b. Functional Evaluation of a De Novo GRIN2A Mutation Identified in a Patient with Profound Global Developmental Delay and Refractory Epilepsy. *Mol Pharmacol,* 91**,** 317-330.

CHEN, X., LONG, F., CAI, B., CHEN, X. & CHEN, G. 2017c. A novel relationship for schizophrenia, bipolar and major depressive disorder Part 5: a hint from chromosome 5 high density association screen. *Am J Transl Res,* 9**,** 2473-2491.

CHIAL, H. 2008. DNA sequencing technologies key to the Human Genome Projec. *Nature Education,* 1(1).

CHIARELLA, S. E., RABIN, E. E., OSTILLA, L. A., FLOZAK, A. S. & GOTTARDI, C. J. 2018. αT-catenin: A developmentally dispensable, disease-linked member of the α-catenin family. *Tissue Barriers,* 6**,** e1463896.

CHO, M. J., KWON, S. S., KO, A., LEE, S. T., LEE, Y. M., KIM, H. D., CHUNG, H. J., KIM, S. H., LEE, J. S., KIM, D. S. & KANG, H. C. 2018. Efficacy of Stiripentol in Dravet Syndrome with or without SCN1A Mutations. *J Clin Neurol,* 14**,** 22-28.

CHOI, Y., SIMS, G. E., MURPHY, S., MILLER, J. R. & CHAN, A. P. 2012. Predicting the functional effect of amino acid substitutions and indels. *PLoS One,* 7**,** e46688.

CHOONG, C. J., BABA, K. & MOCHIZUKI, H. 2016. Gene therapy for neurological disorders. *Expert Opin Biol Ther,* 16**,** 143-59.

CO, M., ANDERSON, A. G. & KONOPKA, G. 2020. FOXP transcription factors in vertebrate brain development, function, and disorders. *Wiley Interdiscip Rev Dev Biol***,** e375.

COCKERELL, I., BOLLING, G. & NAKKEN, K. O. 2011. Landau-Kleffner syndrome in Norway: long-term prognosis and experiences with the health services and educational systems. *Epilepsy Behav,* 21**,** 153-9.

COHEN, D. R., CHENG, C. W., CHENG, S. H. & HUI, C. C. 2000. Expression of two novel mouse Iroquois homeobox genes during neurogenesis. *Mech Dev,* 91**,** 317-21.

COLASANTE, G., QIU, Y., MASSIMINO, L., DI BERARDINO, C., CORNFORD, J. H., SNOWBALL, A., WESTON, M., JONES, S. P., GIANNELLI, S., LIEB, A., SCHORGE, S., KULLMANN, D. M., BROCCOLI, V. & LIGNANI, G. 2020. In vivo CRISPRa decreases seizures and rescues cognitive deficits in a rodent model of epilepsy. *Brain,* 143**,** 891-905.

COLE, A. J., ANDERMANN, F., TAYLOR, L., OLIVIER, A., RASMUSSEN, T., ROBITAILLE, Y. & SPIRE, J. P. 1988. The Landau-Kleffner syndrome of acquired epileptic aphasia: unusual clinical outcome, surgical experience, and absence of encephalitis. *Neurology,* 38**,** 31-8.

COLE, B. A., JOHNSON, R. M., DEJAKAISAYA, H., PILATI, N., FISHWICK, C. W. G., MUENCH, S. P. & LIPPIAT, J. D. 2020. Structure-Based Identification and Characterization of Inhibitors of the Epilepsy-Associated K(Na)1.1 (KCNT1) Potassium Channel. *iScience,* 23**,** 101100.

COLIN, E., DANIEL, J., ZIEGLER, A., WAKIM, J., SCRIVO, A., HAACK, T. B., KHIATI, S., DENOMME, A. S., AMATI-BONNEAU, P., CHARIF, M., PROCACCIO, V., REYNIER, P., ALECK, K. A., BOTTO, L. D., HERPER, C. L., KAISER, C. S., NABBOUT, R., N'GUYEN, S., MORA-LORCA, J. A., ASSMANN, B., CHRIST, S., MEITINGER, T., STROM, T. M., PROKISCH, H., MIRANDA-VIZUETE, A., HOFFMANN, G. F., LENAERS, G., BOMONT, P., LIEBAU, E. & BONNEAU, D. 2016. Biallelic Variants in UBA5 Reveal that Disruption of the UFM1 Cascade Can Result in Early-Onset Encephalopathy. *Am J Hum Genet,* 99**,** 695-703.

COLLINS, A. L., MA, D., WHITEHEAD, P. L., MARTIN, E. R., WRIGHT, H. H., ABRAMSON, R. K., HUSSMAN, J. P., HAINES, J. L., CUCCARO, M. L., GILBERT, J. R. & PERICAK-

VANCE, M. A. 2006. Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. *Neurogenetics,* 7**,** 167-74.

CONNOLLY, A. M., CHEZ, M., STREIF, E. M., KEELING, R. M., GOLUMBEK, P. T., KWON, J. M., RIVIELLO, J. J., ROBINSON, R. G., NEUMAN, R. J. & DEUEL, R. M. 2006. Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. *Biol Psychiatry,* 59**,** 354-63.

CONNOLLY, A. M., CHEZ, M. G., PESTRONK, A., ARNOLD, S. T., MEHTA, S. & DEUEL, R. K. 1999. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. *J Pediatr,* 134**,** 607-13.

CONROY, J., ALLEN, N. M., GORMAN, K., O'HALLORAN, E., SHAHWAN, A., LYNCH, B., LYNCH, S. A., ENNIS, S. & KING, M. D. 2016. Novel European SLC1A4 variant: infantile spasms and population ancestry analysis. *J Hum Genet,* 61**,** 761-4.

CONROY, J., MCGETTIGAN, P. A., MCCREARY, D., SHAH, N., COLLINS, K., PARRY-FIELDER, B., MORAN, M., HANRAHAN, D., DEONNA, T. W., KORFF, C. M., WEBB, D., ENNIS, S., LYNCH, S. A. & KING, M. D. 2014. Towards the identification of a genetic basis for Landau-Kleffner syndrome. *Epilepsia,* 55**,** 858-65.

CONTI, V., ARACRI, P., CHITI, L., BRUSCO, S., MARI, F., MARINI, C., ALBANESE, M., MARCHI, A., LIGUORI, C., PLACIDI, F., ROMIGI, A., BECCHETTI, A. & GUERRINI, R. 2015. Nocturnal frontal lobe epilepsy with paroxysmal arousals due to CHRNA2 loss of function. *Neurology,* 84**,** 1520-8.

CORBETT, M. A., BELLOWS, S. T., LI, M., CARROLL, R., MICALLEF, S., CARVILL, G. L., MYERS, C. T., HOWELL, K. B., MALJEVIC, S., LERCHE, H., GAZINA, E. V., MEFFORD, H. C., BAHLO, M., BERKOVIC, S. F., PETROU, S., SCHEFFER, I. E. & GECZ, J. 2016. Dominant KCNA2 mutation causes episodic ataxia and pharmacoresponsive epilepsy. *Neurology,* 87**,** 1975-1984.

CORBETT, M. A., SCHWAKE, M., BAHLO, M., DIBBENS, L. M., LIN, M., GANDOLFO, L. C., VEARS, D. F., O'SULLIVAN, J. D., ROBERTSON, T., BAYLY, M. A., GARDNER, A. E., VLAAR, A. M., KORENKE, G. C., BLOEM, B. R., DE COO, I. F., VERHAGEN, J. M., LEHESJOKI, A. E., GECZ, J. & BERKOVIC, S. F. 2011. A mutation in the Golgi Qb-SNARE gene GOSR2 causes progressive myoclonus epilepsy with early ataxia. *Am J Hum Genet,* 88**,** 657-63.

COURCET, J. B., FAIVRE, L., MALZAC, P., MASUREL-PAULET, A., LOPEZ, E., CALLIER, P., LAMBERT, L., LEMESLE, M., THEVENON, J., GIGOT, N., DUPLOMB, L., RAGON, C., MARLE, N., MOSCA-BOIDRON, A. L., HUET, F., PHILIPPE, C., MONCLA, A. & THAUVIN-ROBINET, C. 2012. The DYRK1A gene is a cause of syndromic intellectual disability with severe microcephaly and epilepsy. *J Med Genet,* 49**,** 731-6.

CROSS, J. H. & NEVILLE, B. G. 2009. The surgical treatment of Landau-Kleffner syndrome. *Epilepsia,* 50 Suppl 7**,** 63-7.

CURATOLO, P., NABBOUT, R., LAGAE, L., ARONICA, E., FERREIRA, J. C., FEUCHT, M., HERTZBERG, C., JANSEN, A. C., JANSEN, F., KOTULSKA, K., MOAVERO, R., O'CALLAGHAN, F., PAPAVASILIOU, A., TZADOK, M. & JÓŹWIAK, S. 2018. Management of epilepsy associated with tuberous sclerosis complex: Updated clinical recommendations. *Eur J Paediatr Neurol,* 22**,** 738-748.

DABELL, M. P., ROSENFELD, J. A., BADER, P., ESCOBAR, L. F., EL-KHECHEN, D., VALLEE, S. E., DINULOS, M. B., CURRY, C., FISHER, J., TERVO, R., HANNIBAL, M. C., SIEFKAS, K., WYATT, P. R., HUGHES, L., SMITH, R., ELLINGWOOD, S., LACASSIE, Y., STROUD, T., FARRELL, S. A., SANCHEZ-LARA, P. A., RANDOLPH, L. M., NIYAZOV, D., STEVENS, C. A., SCHOONVELD, C., SKIDMORE, D., MACKAY, S., MILES, J. H., MOODLEY, M., HUILLET, A., NEILL, N. J., ELLISON, J. W., BALLIF, B. C. & SHAFFER, L. G. 2013. Investigation of NRXN1 deletions: clinical and molecular characterization. *Am J Med Genet A,* 161a**,** 717-31.

DANTI, F. R., GALOSI, S., ROMANI, M., MONTOMOLI, M., CARSS, K. J., RAYMOND, F. L., PARRINI, E., BIANCHINI, C., MCSHANE, T., DALE, R. C., MOHAMMAD, S. S., SHAH, U., MAHANT, N., NG, J., MCTAGUE, A., SAMANTA, R., VADLAMANI, G., VALENTE, E. M., LEUZZI, V., KURIAN, M. A. & GUERRINI, R. 2017. GNAO1 encephalopathy: Broadening the phenotype and evaluating treatment and outcome. *Neurol Genet,* 3**,** e143.

DAWSON, R. E., NIETO GUIL, A. F., ROBERTSON, L. J., PILTZ, S. G., HUGHES, J. N. & THOMAS, P. Q. 2020. Functional screening of GATOR1 complex variants reveals a role for mTORC1 deregulation in FCD and focal epilepsy. *Neurobiol Dis,* 134**,** 104640.

DAZZO, E., FANCIULLI, M., SERIOLI, E., MINERVINI, G., PULITANO, P., BINELLI, S., DI BONAVENTURA, C., LUISI, C., PASINI, E., STRIANO, S., STRIANO, P., COPPOLA, G., CHIAVEGATO, A., RADOVIC, S., SPADOTTO, A., UZZAU, S., LA NEVE, A., GIALLONARDO, A. T., MECARELLI, O., TOSATTO, S. C., OTTMAN, R., MICHELUCCI, R. & NOBILE, C. 2015. Heterozygous reelin mutations cause autosomal-dominant lateral temporal epilepsy. *Am J Hum Genet,* 96**,** 992-1000.

DE KOVEL, C. G., BRILSTRA, E. H., VAN KEMPEN, M. J., VAN'T SLOT, R., NIJMAN, I. J., AFAWI, Z., DE JONGHE, P., DJEMIE, T., GUERRINI, R., HARDIES, K., HELBIG, I., HENDRICKX, R., KANAAN, M., KRAMER, U., LEHESJOKI, A. E., LEMKE, J. R., MARINI, C., MEI, D., MOLLER, R. S., PENDZIWIAT, M., STAMBERGER, H., SULS, A., WECKHUYSEN, S. & KOELEMAN, B. P. 2016. Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. *Mol Genet Genomic Med,* 4**,** 568-80.

DE LANGE, I. M., GUNNING, B., SONSMA, A. C. M., VAN GEMERT, L., VAN KEMPEN, M., VERBEEK, N. E., NICOLAI, J., KNOERS, N., KOELEMAN, B. P. C. & BRILSTRA, E. H. 2018. Influence of contraindicated medication use on cognitive outcome in Dravet syndrome and age at first afebrile seizure as a clinical predictor in SCN1A-related seizure phenotypes. *Epilepsia,* 59**,** 1154-1165.

DE LIGT, J., WILLEMSEN, M. H., VAN BON, B. W., KLEEFSTRA, T., YNTEMA, H. G., KROES, T., VULTO-VAN SILFHOUT, A. T., KOOLEN, D. A., DE VRIES, P., GILISSEN, C., DEL

ROSARIO, M., HOISCHEN, A., SCHEFFER, H., DE VRIES, B. B., BRUNNER, H. G., VELTMAN, J. A. & VISSERS, L. E. 2012. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med,* 367**,** 1921-9.

DE NEGRI, M., BAGLIETTO, M. G., BATTAGLIA, F. M., GAGGERO, R., PESSAGNO, A. & RECANATI, L. 1995. Treatment of electrical status epilepticus by short diazepam (DZP) cycles after DZP rectal bolus test. *Brain Dev,* 17**,** 330-3.

DEAN, L. 2012. Pitt-Hopkins Syndrome. *In:* PRATT, V., MCLEOD, H., RUBINSTEIN, W., DEAN, L. & MALHEIRO, A. (eds.) *Medical Genetics Summaries.* Bethesda (MD): National Center for Biotechnology Information (US).

DEDEK, K., FUSCO, L., TELOY, N. & STEINLEIN, O. K. 2003. Neonatal convulsions and epileptic encephalopathy in an Italian family with a missense mutation in the fifth transmembrane region of KCNQ2. *Epilepsy Res,* 54**,** 21-7.

DEJANOVIC, B., DJEMIE, T., GRUNEWALD, N., SULS, A., KRESS, V., HETSCH, F., CRAIU, D., ZEMEL, M., GORMLEY, P., LAL, D., MYERS, C. T., MEFFORD, H. C., PALOTIE, A., HELBIG, I., MEIER, J. C., DE JONGHE, P., WECKHUYSEN, S. & SCHWARZ, G. 2015. Simultaneous impairment of neuronal and metabolic function of mutated gephyrin in a patient with epileptic encephalopathy. *EMBO Mol Med,* 7**,** 1580-94.

DELGADO-ESCUETA, A. V., KOELEMAN, B. P., BAILEY, J. N., MEDINA, M. T. & DURON, R. M. 2013. The quest for juvenile myoclonic epilepsy genes. *Epilepsy Behav,* 28 Suppl 1**,** S52-7.

DEONNA, T., BEAUMANOIR, A., GAILLARD, F. & ASSAL, G. 1977. Acquired aphasia in childhood with seizure disorder: a heterogeneous syndrome. *Neuropadiatrie,* 8**,** 263-73.

DEONNA, T., PETER, C. & ZIEGLER, A. L. 1989. Adult follow-up of the acquired aphasia-epilepsy syndrome in childhood. Report of 7 cases. *Neuropediatrics,* 20**,** 132-8.

DEPIENNE, C., BOUTEILLER, D., KEREN, B., CHEURET, E., POIRIER, K., TROUILLARD, O., BENYAHIA, B., QUELIN, C., CARPENTIER, W., JULIA, S., AFENJAR, A., GAUTIER, A., RIVIER, F., MEYER, S., BERQUIN, P., HELIAS, M., PY, I., RIVERA, S., BAHI-BUISSON, N., GOURFINKEL-AN, I., CAZENEUVE, C., RUBERG, M., BRICE, A., NABBOUT, R. & LEGUERN, E. 2009. Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. *PLoS Genet,* 5**,** e1000381.

DEPIENNE, C., TROUILLARD, O., GOURFINKEL-AN, I., SAINT-MARTIN, C., BOUTEILLER, D., GRABER, D., BARTHEZ-CARPENTIER, M. A., GAUTIER, A., VILLENEUVE, N., DRAVET, C., LIVET, M. O., RIVIER-RINGENBACH, C., ADAM, C., DUPONT, S., BAULAC, S., HERON, D., NABBOUT, R. & LEGUERN, E. 2010. Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. *J Med Genet,* 47**,** 404-10.

DEVERMAN, B. E., RAVINA, B. M., BANKIEWICZ, K. S., PAUL, S. M. & SAH, D. W. Y. 2018. Gene therapy for neurological disorders: progress and prospects. *Nat Rev Drug Discov,* 17**,** 641-659.

DEVINSKY, O., VEZZANI, A., O'BRIEN, T. J., JETTE, N., SCHEFFER, I. E., DE CURTIS, M. & PERUCCA, P. 2018. Epilepsy. *Nat Rev Dis Primers,* 4**,** 18024.

DEVRIES, S. P. & PATEL, A. D. 2013. Two patients with a GRIN2A mutation and childhood-onset epilepsy. *Pediatr Neurol,* 49**,** 482-5.

DHARMADHIKARI, A. V., KANG, S. H., SZAFRANSKI, P., PERSON, R. E., SAMPATH, S., PRAKASH, S. K., BADER, P. I., PHILLIPS, J. A., 3RD, HANNIG, V., WILLIAMS, M., VINSON, S. S., WILFONG, A. A., REIMSCHISEL, T. E., CRAIGEN, W. J., PATEL, A., BI, W., LUPSKI, J. R., BELMONT, J., CHEUNG, S. W. & STANKIEWICZ, P. 2012. Small rare recurrent deletions and reciprocal duplications in 2q21.1, including brain-specific ARHGEF4 and GPR148. *Hum Mol Genet,* 21**,** 3345-55.

DIBBENS, L. M., FENG, H. J., RICHARDS, M. C., HARKIN, L. A., HODGSON, B. L., SCOTT, D., JENKINS, M., PETROU, S., SUTHERLAND, G. R., SCHEFFER, I. E., BERKOVIC, S. F., MACDONALD, R. L. & MULLEY, J. C. 2004. GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet,* 13**,** 1315-9.

DIXON, C. L., ZHANG, Y. & LYNCH, J. W. 2015. Generation of Functional Inhibitory Synapses Incorporating Defined Combinations of GABA(A) or Glycine Receptor Subunits. *Front Mol Neurosci,* 8**,** 80.

DOWNES, M., GREENAWAY, R., CLARK, M., HELEN CROSS, J., JOLLEFF, N., HARKNESS, W., KALIAKATSOS, M., BOYD, S., WHITE, S. & NEVILLE, B. G. 2015. Outcome following multiple subpial transection in Landau-Kleffner syndrome and related regression. *Epilepsia,* 56**,** 1760-6.

DRAGICH, J. M., KUWAJIMA, T., HIROSE-IKEDA, M., YOON, M. S., EENJES, E., BOSCO, J. R., FOX, L. M., LYSTAD, A. H., OO, T. F., YARYGINA, O. & MITA, T. 2016a. Autophagy linked FYVE (Alfy/WDFY3) is required for establishing neuronal connectivity in the mammalian brain. 5.

DRAGICH, J. M., KUWAJIMA, T., HIROSE-IKEDA, M., YOON, M. S., EENJES, E., BOSCO, J. R., FOX, L. M., LYSTAD, A. H., OO, T. F., YARYGINA, O., MITA, T., WAGURI, S., ICHIMURA, Y., KOMATSU, M., SIMONSEN, A., BURKE, R. E., MASON, C. A. & YAMAMOTO, A. 2016b. Autophagy linked FYVE (Alfy/WDFY3) is required for establishing neuronal connectivity in the mammalian brain. *Elife,* 5.

DULAC, O., BILLARD, C. & ARTHUIS, M. 1983. [Electroclinical and developmental aspects of epilepsy in the aphasia-epilepsy syndrome]. *Arch Fr Pediatr,* 40**,** 299-308.

DURAN, M. H., GUIMARÃES, C. A., MEDEIROS, L. L. & GUERREIRO, M. M. 2009. Landau-Kleffner syndrome: long-term follow-up. *Brain Dev,* 31**,** 58-63.

DURU, N., ISERI, S. A., SELCUK, N. & TOLUN, A. 2010. Early-onset progressive myoclonic epilepsy with dystonia mapping to 16pter-p13.3. *J Neurogenet,* 24**,** 207-15.

DWIVEDI, R., RAMANUJAM, B., CHANDRA, P. S., SAPRA, S., GULATI, S., KALAIVANI, M., GARG, A., BAL, C. S., TRIPATHI, M., DWIVEDI, S. N., SAGAR, R., SARKAR, C. & TRIPATHI, M. 2017. Surgery for Drug-Resistant Epilepsy in Children. *N Engl J Med,* 377**,** 1639-1647.

DYMENT, D. A., TETREAULT, M., BEAULIEU, C. L., HARTLEY, T., FERREIRA, P., CHARDON, J. W., MARCADIER, J., SAWYER, S. L., MOSCA, S. J., INNES, A. M., PARBOOSINGH, J. S., BULMAN, D. E., SCHWARTZENTRUBER, J., MAJEWSKI, J., TARNOPOLSKY, M. & BOYCOTT, K. M. 2015. Whole-exome sequencing broadens the phenotypic spectrum of rare pediatric epilepsy: a retrospective study. *Clin Genet,* 88**,** 34-40.

EBACH, K., JOOS, H., DOOSE, H., STEPHANI, U., KURLEMANN, G., FIEDLER, B., HAHN, A., HAUSER, E., HUNDT, K., HOLTHAUSEN, H., MULLER, U. & NEUBAUER, B. A. 2005. SCN1A mutation analysis in myoclonic astatic epilepsy and severe idiopathic generalized epilepsy of infancy with generalized tonic-clonic seizures. *Neuropediatrics,* 36**,** 210-3.

EBRAHIMI-FAKHARI, D., SAFFARI, A., WESTENBERGER, A. & KLEIN, C. 2015. The evolving spectrum of PRRT2-associated paroxysmal diseases. *Brain,* 138**,** 3476-95.

EDVARDSON, S., BAUMANN, A. M., MUHLENHOFF, M., STEPHAN, O., KUSS, A. W., SHAAG, A., HE, L., ZENVIRT, S., TANZI, R., GERARDY-SCHAHN, R. & ELPELEG, O. 2013. West syndrome caused by ST3Gal-III deficiency. *Epilepsia,* 54**,** e24-7.

EDVARDSON, S., WANG, H., DOR, T., ATAWNEH, O., YAACOV, B., GARTNER, J., CINNAMON, Y., CHEN, S. & ELPELEG, O. 2016. Microcephaly-dystonia due to mutated PLEKHG2 with impaired actin polymerization. *Neurogenetics,* 17**,** 25-30.

ENGEL, J., JR. 2001. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia,* 42**,** 796-803.

ENGEL, J., JR. 2006. Report of the ILAE classification core group. *Epilepsia,* 47**,** 1558-68.

ENGEL, J., JR., MCDERMOTT, M. P., WIEBE, S., LANGFITT, J. T., STERN, J. M., DEWAR, S., SPERLING, M. R., GARDINER, I., ERBA, G., FRIED, I., JACOBS, M., VINTERS, H. V., MINTZER, S., KIEBURTZ, K. & EARLY RANDOMIZED SURGICAL EPILEPSY TRIAL STUDY, G. 2012. Early surgical therapy for drug-resistant temporal lobe epilepsy: a randomized trial. *JAMA,* 307**,** 922-30.

EPI4K CONSORTIUM 2016. De Novo Mutations in SLC1A2 and CACNA1A Are Important Causes of Epileptic Encephalopathies. *Am J Hum Genet,* 99**,** 287-98.

EPI4K CONSORTIUM, ALLEN, A. S., BERKOVIC, S. F., COSSETTE, P., DELANTY, N., DLUGOS, D., EICHLER, E. E., EPSTEIN, M. P., GLAUSER, T., GOLDSTEIN, D. B., HAN, Y., HEINZEN, E. L., HITOMI, Y., HOWELL, K. B., JOHNSON, M. R., KUZNIECKY, R., LOWENSTEIN, D. H., LU, Y. F., MADOU, M. R., MARSON, A. G., MEFFORD, H. C., ESMAEELI NIEH, S., O'BRIEN, T. J., OTTMAN, R., PETROVSKI, S., PODURI, A., RUZZO, E. K., SCHEFFER, I. E., SHERR, E. H., YUSKAITIS, C. J., ABOU-KHALIL, B., ALLDREDGE, B. K., BAUTISTA, J. F., BERKOVIC, S. F., BORO, A., CASCINO, G. D., CONSALVO, D., CRUMRINE, P., DEVINSKY, O., DLUGOS, D., EPSTEIN, M. P., FIOL,

M., FOUNTAIN, N. B., FRENCH, J., FRIEDMAN, D., GELLER, E. B., GLAUSER, T., GLYNN, S., HAUT, S. R., HAYWARD, J., HELMERS, S. L., JOSHI, S., KANNER, A., KIRSCH, H. E., KNOWLTON, R. C., KOSSOFF, E. H., KUPERMAN, R., KUZNIECKY, R., LOWENSTEIN, D. H., MCGUIRE, S. M., MOTIKA, P. V., NOVOTNY, E. J., OTTMAN, R., PAOLICCHI, J. M., PARENT, J. M., PARK, K., PODURI, A., SCHEFFER, I. E., SHELLHAAS, R. A., SHERR, E. H., SHIH, J. J., SINGH, R., SIRVEN, J., SMITH, M. C., SULLIVAN, J., LIN THIO, L., VENKAT, A., VINING, E. P., VON ALLMEN, G. K., WEISENBERG, J. L., WIDDESS-WALSH, P. & WINAWER, M. R. 2013. De novo mutations in epileptic encephalopathies. *Nature,* 501**,** 217-21.

EPILEPSY PHENOME/GENOME PROJECT & EPI4K CONSORTIUM 2015. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. *Ann Neurol,* 78**,** 323-8.

EREN, E. & ÖZÖREN, N. 2019. The NLRP3 inflammasome: a new player in neurological diseases. *Turk J Biol,* 43**,** 349-359.

ESCAYG, A. & GOLDIN, A. L. 2010. Sodium channel SCN1A and epilepsy: mutations and mechanisms. *Epilepsia,* 51**,** 1650-8.

ESPOSITO, S., PRISTERA, A., MARESCA, G., CAVALLARO, S., FELSANI, A., FLORENZANO, F., MANNI, L., CIOTTI, M. T., POLLEGIONI, L., BORSELLO, T. & CANU, N. 2012. Contribution of serine racemase/d-serine pathway to neuronal apoptosis. *Aging Cell,* 11**,** 588-98.

ESTRUCH, S. B., GRAHAM, S. A., CHINNAPPA, S. M., DERIZIOTIS, P. & FISHER, S. E. 2016. Functional characterization of rare FOXP2 variants in neurodevelopmental disorder. *J Neurodev Disord,* 8**,** 44.

EUROEPINOMICS-RES CONSORTIUM, EPILEPSY PHENOME/GENOME PROJECT & EPI4K CONSORTIUM 2014. De novo mutations in synaptic transmission genes including DNM1 cause epileptic encephalopathies. *Am J Hum Genet,* 95**,** 360-70.

FAINBERG, N., HARPER, A., TCHAPYJNIKOV, D. & MIKATI, M. A. 2016. Response to immunotherapy in a patient with Landau-Kleffner syndrome and GRIN2A mutation. *Epileptic Disord,* 18**,** 97-100.

FALK, M. J., LI, D., GAI, X., MCCORMICK, E., PLACE, E., LASORSA, F. M., OTIENO, F. G., HOU, C., KIM, C. E., ABDEL-MAGID, N., VAZQUEZ, L., MENTCH, F. D., CHIAVACCI, R., LIANG, J., LIU, X., JIANG, H., GIANNUZZI, G., MARSH, E. D., YIRAN, G., TIAN, L., PALMIERI, F. & HAKONARSON, H. 2014. AGC1 Deficiency Causes Infantile Epilepsy, Abnormal Myelination, and Reduced N-Acetylaspartate. *JIMD Rep,* 14**,** 77-85.

FANDINO, M., CONNOLLY, M., USHER, L., PALM, S. & KOZAK, F. K. 2011. Landau-Kleffner syndrome: a rare auditory processing disorder series of cases and review of the literature. *Int J Pediatr Otorhinolaryngol,* 75**,** 33-8.

FARNAES, L., NAHAS, S. A., CHOWDHURY, S., NELSON, J., BATALOV, S., DIMMOCK, D. M. & KINGSMORE, S. F. 2017. Rapid whole-genome sequencing identifies a novel

GABRA1 variant associated with West syndrome. *Cold Spring Harb Mol Case Stud,* 3.

FASSIO, A., ESPOSITO, A., KATO, M., SAITSU, H., MEI, D., MARINI, C., CONTI, V., NAKASHIMA, M., OKAMOTO, N., OLMEZ TURKER, A., ALBUZ, B., SEMERCI GUNDUZ, C. N., YANAGIHARA, K., BELMONTE, E., MARAGLIANO, L., RAMSEY, K., BALAK, C., SINIARD, A., NARAYANAN, V., OHBA, C., SHIINA, M., OGATA, K., MATSUMOTO, N., BENFENATI, F. & GUERRINI, R. 2018. De novo mutations of the ATP6V1A gene cause developmental encephalopathy with epilepsy. *Brain,* 141**,** 1703-1718.

FAYAD, M. N., CHOUEIRI, R. & MIKATI, M. 1997. Landau-Kleffner syndrome: consistent response to repeated intravenous gamma-globulin doses: a case report. *Epilepsia,* 38**,** 489-94.

FEHR, S., WILSON, M., DOWNS, J., WILLIAMS, S., MURGIA, A., SARTORI, S., VECCHI, M., HO, G., POLLI, R., PSONI, S., BAO, X., DE KLERK, N., LEONARD, H. & CHRISTODOULOU, J. 2013. The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. *Eur J Hum Genet,* 21**,** 266-73.

FERNANDES, I. R., CRUZ, A. C. P., FERRASA, A., PHAN, D., HERAI, R. H. & MUOTRI, A. R. 2018. Genetic variations on SETD5 underlying autistic conditions. *Dev Neurobiol,* 78**,** 500-518.

FERNÁNDEZ, I. S., CHAPMAN, K. E., PETERS, J. M., KOTHARE, S. V., NORDLI, D. R., JR., JENSEN, F. E., BERG, A. T. & LODDENKEMPER, T. 2013. The tower of Babel: survey on concepts and terminology in electrical status epilepticus in sleep and continuous spikes and waves during sleep in North America. *Epilepsia,* 54**,** 741-50.

FERNANDEZ-MARMIESSE, A., KUSUMOTO, H., REKARTE, S., ROCA, I., ZHANG, J., MYERS, S. J., TRAYNELIS, S. F., COUCE, M. L., GUTIERREZ-SOLANA, L. & YUAN, H. 2018. A novel missense mutation in GRIN2A causes a nonepileptic neurodevelopmental disorder. *Mov Disord*.

FIORE, A., LIANG, Y., LIN, Y. H., TUNG, J., WANG, H., LANGLAIS, D. & NIJNIK, A. 2020. Deubiquitinase MYSM1 in the Hematopoietic System and beyond: A Current Review. *Int J Mol Sci,* 21.

FITZGERALD, M. P., FIANNACCA, M., SMITH, D. M., GERTLER, T. S., GUNNING, B., SYRBE, S., VERBEEK, N., STAMBERGER, H., WECKHUYSEN, S., CEULEMANS, B., SCHOONJANS, A. S., ROSSI, M., DEMARQUAY, G., LESCA, G., OLOFSSON, K., KOOLEN, D. A., HORNEMANN, F., BAULAC, S., RUBBOLI, G., MINKS, K. Q., LEE, B., HELBIG, I., DLUGOS, D., MØLLER, R. S. & BEARDEN, D. 2019. Treatment Responsiveness in KCNT1-Related Epilepsy. *Neurotherapeutics,* 16**,** 848-857.

FOLMSBEE, S. S., WILCOX, D. R., TYBERGHEIN, K., DE BLESER, P., TOURTELLOTTE, W. G., VAN HENGEL, J., VAN ROY, F. & GOTTARDI, C. J. 2016. αT-catenin in restricted brain cell types and its potential connection to autism. *J Mol Psychiatry,* 4**,** 2.

FRANZ, D. N., LAWSON, J. A., YAPICI, Z., IKEDA, H., POLSTER, T., NABBOUT, R., CURATOLO, P., DE VRIES, P. J., DLUGOS, D. J., VOI, M., FAN, J., VAURY, A., PELOV, D. & FRENCH, J. A. 2018. Everolimus for treatment-refractory seizures in TSC: Extension of a randomized controlled trial. *Neurol Clin Pract,* 8**,** 412-420.

FRENCH, J. A., LAWSON, J. A., YAPICI, Z., IKEDA, H., POLSTER, T., NABBOUT, R., CURATOLO, P., DE VRIES, P. J., DLUGOS, D. J., BERKOWITZ, N., VOI, M., PEYRARD, S., PELOV, D. & FRANZ, D. N. 2016. 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,* 388**,** 2153-2163.

FRIKSTAD, K. M., MOLINARI, E., THORESEN, M., RAMSBOTTOM, S. A., HUGHES, F., LETTEBOER, S. J. F., GILANI, S., SCHINK, K. O., STOKKE, T., GEIMER, S., PEDERSEN, L. B., GILES, R. H., AKHMANOVA, A., ROEPMAN, R., SAYER, J. A. & PATZKE, S. 2019. A CEP104-CSPP1 Complex Is Required for Formation of Primary Cilia Competent in Hedgehog Signaling. *Cell Rep,* 28**,** 1907-1922.e6.

FU, Y., WU, Z., GUO, Z., CHEN, L., MA, Y., WANG, Z., XIAO, W. & WANG, Y. 2020. Systems-level analysis identifies key regulators driving epileptogenesis in temporal lobe epilepsy. *Genomics,* 112**,** 1768-1780.

FUJIWARA, T., SUGAWARA, T., MAZAKI-MIYAZAKI, E., TAKAHASHI, Y., FUKUSHIMA, K., WATANABE, M., HARA, K., MORIKAWA, T., YAGI, K., YAMAKAWA, K. & INOUE, Y. 2003. Mutations of sodium channel alpha subunit type 1 (SCN1A) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures. *Brain,* 126**,** 531-46.

FUKUOKA, M., KUKI, I., KAWAWAKI, H., OKAZAKI, S., KIM, K., HATTORI, Y., TSUJI, H., NUKUI, M., INOUE, T., YOSHIDA, Y., UDA, T., KIMURA, S., MOGAMI, Y., SUZUKI, Y., OKAMOTO, N., SAITSU, H. & MATSUMOTO, N. 2016. Quinidine therapy for West syndrome with KCNTI mutation: A case report. *Brain Dev*.

GADEA, G. & BLANGY, A. 2014. Dock-family exchange factors in cell migration and disease. *Eur J Cell Biol,* 93**,** 466-77.

GAO, K., TANKOVIC, A., ZHANG, Y., KUSUMOTO, H., ZHANG, J., CHEN, W., XIANGWEI, W., SHAULSKY, G. H., HU, C., TRAYNELIS, S. F., YUAN, H. & JIANG, Y. 2017. A de novo loss-of-function GRIN2A mutation associated with childhood focal epilepsy and acquired epileptic aphasia. *PLoS One,* 12**,** e0170818.

GASCON, G., VICTOR, D. & LOMBROSO, C. T. 1973. Language disorders, convulsive disorder, and electroencephalographic abnormalities. Acquired syndrome in children. *Arch Neurol,* 28**,** 156-62.

GASSMANN, M., SHABAN, H., VIGOT, R., SANSIG, G., HALLER, C., BARBIERI, S., HUMEAU, Y., SCHULER, V., MULLER, M., KINZEL, B., KLEBS, K., SCHMUTZ, M., FROESTL, W., HEID, J., KELLY, P. H., GENTRY, C., JATON, A. L., VAN DER PUTTEN, H., MOMBEREAU, C., LECOURTIER, L., MOSBACHER, J., CRYAN, J. F., FRITSCHY, J. M., LUTHI, A., KAUPMANN, K. & BETTLER, B. 2004. Redistribution of GABAB(1)

protein and atypical GABAB responses in GABAB(2)-deficient mice. *J Neurosci,* 24**,** 6086-97.

GENTON, P., MATON, B., OGIHARA, M., SAMOGGIA, G., GUERRINI, R., MEDINA, M. T., DRAVET, C. & ROGER, J. 1992. Continuous focal spikes during REM sleep in a case of acquired aphasia (Landau-Kleffner syndrome). *Sleep,* 15**,** 454-60.

GORMAN, K. M., FORMAN, E., CONROY, J., ALLEN, N. M., SHAHWAN, A., LYNCH, S. A., ENNIS, S. & KING, M. D. 2017. Novel SMC1A variant and epilepsy of infancy with migrating focal seizures: Expansion of the phenotype. *Epilepsia,* 58**,** 1301-1302.

GRIGGS, B. L., LADD, S., SAUL, R. A., DUPONT, B. R. & SRIVASTAVA, A. K. 2008. Dedicator of cytokinesis 8 is disrupted in two patients with mental retardation and developmental disabilities. *Genomics,* 91**,** 195-202.

GROTE, C. L., VAN SLYKE, P. & HOEPPNER, J. A. 1999. Language outcome following multiple subpial transection for Landau-Kleffner syndrome. *Brain,* 122 (Pt 3)**,** 561-6.

GU, T., ZHAO, T., KOHLI, U. & HEWES, R. S. 2017. The large and small SPEN family proteins stimulate axon outgrowth during neurosecretory cell remodeling in Drosophila. *Dev Biol,* 431**,** 226-238.

GUELLA, I., HUH, L., MCKENZIE, M. B., TOYOTA, E. B., BEBIN, E. M., THOMPSON, M. L., COOPER, G. M., EVANS, D. M., BUERKI, S. E., ADAM, S., VAN ALLEN, M. I., NELSON, T. N., CONNOLLY, M. B., FARRER, M. J. & DEMOS, M. 2016. De novo FGF12 mutation in 2 patients with neonatal-onset epilepsy. *Neurol Genet,* 2**,** e120.

GUELLA, I., MCKENZIE, M. B., EVANS, D. M., BUERKI, S. E., TOYOTA, E. B., VAN ALLEN, M. I., SURI, M., ELMSLIE, F., SIMON, M. E. H., VAN GASSEN, K. L. I., HERON, D., KEREN, B., NAVA, C., CONNOLLY, M. B., DEMOS, M. & FARRER, M. J. 2017. De Novo Mutations in YWHAG Cause Early-Onset Epilepsy. *Am J Hum Genet,* 101**,** 300-310.

GUERRINI, R., SHANAHAN, J. L., CARROZZO, R., BONANNI, P., HIGGS, D. R. & GIBBONS, R. J. 2000. A nonsense mutation of the ATRX gene causing mild mental retardation and epilepsy. *Ann Neurol,* 47**,** 117-21.

GUO, H., WANG, T., WU, H., LONG, M., COE, B. P., LI, H., XUN, G., OU, J., CHEN, B., DUAN, G., BAI, T., ZHAO, N., SHEN, Y., LI, Y., WANG, Y., ZHANG, Y., BAKER, C., LIU, Y., PANG, N., HUANG, L., HAN, L., JIA, X., LIU, C., NI, H., YANG, X., XIA, L., CHEN, J., SHEN, L., LI, Y., ZHAO, R., ZHAO, W., PENG, J., PAN, Q., LONG, Z., SU, W., TAN, J., DU, X., KE, X., YAO, M., HU, Z., ZOU, X., ZHAO, J., BERNIER, R. A., EICHLER, E. E. & XIA, K. 2018. Inherited and multiple de novo mutations in autism/developmental delay risk genes suggest a multifactorial model. *Mol Autism,* 9**,** 64.

GURNEY, M. E. 2019. Genetic Association of Phosphodiesterases With Human Cognitive Performance. *Front Mol Neurosci,* 12**,** 22.

GURURAJ, S., PALMER, E. E., SHEEHAN, G. D., KANDULA, T., MACINTOSH, R., YING, K., MORRIS, P., TAO, J., DIAS, K. R., ZHU, Y., DINGER, M. E., COWLEY, M. J., KIRK, E. P., ROSCIOLI, T., SACHDEV, R., DUFFEY, M. E., BYE, A. & BHATTACHARJEE, A. 2017. A De Novo Mutation in the Sodium-Activated Potassium Channel KCNT2 Alters Ion Selectivity and Causes Epileptic Encephalopathy. *Cell Rep,* 21**,** 926-933.

HACKOS, D. H., LUPARDUS, P. J., GRAND, T., CHEN, Y., WANG, T. M., REYNEN, P., GUSTAFSON, A., WALLWEBER, H. J., VOLGRAF, M., SELLERS, B. D., SCHWARZ, J. B., PAOLETTI, P., SHENG, M., ZHOU, Q. & HANSON, J. E. 2016. Positive Allosteric Modulators of GluN2A-Containing NMDARs with Distinct Modes of Action and Impacts on Circuit Function. *Neuron,* 89**,** 983-99.

HAMANN, J., AUST, G., ARAÇ, D., ENGEL, F. B., FORMSTONE, C., FREDRIKSSON, R., HALL, R. A., HARTY, B. L., KIRCHHOFF, C., KNAPP, B., KRISHNAN, A., LIEBSCHER, I., LIN, H. H., MARTINELLI, D. C., MONK, K. R., PEETERS, M. C., PIAO, X., PRÖMEL, S., SCHÖNEBERG, T., SCHWARTZ, T. W., SINGER, K., STACEY, M., USHKARYOV, Y. A., VALLON, M., WOLFRUM, U., WRIGHT, M. W., XU, L., LANGENHAN, T. & SCHIÖTH, H. B. 2015. International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol Rev,* 67**,** 338-67.

HAMDAN, F. F., GAUTHIER, J., ARAKI, Y., LIN, D. T., YOSHIZAWA, Y., HIGASHI, K., PARK, A. R., SPIEGELMAN, D., DOBRZENIECKA, S., PITON, A., TOMITORI, H., DAOUD, H., MASSICOTTE, C., HENRION, E., DIALLO, O., SHEKARABI, M., MARINEAU, C., SHEVELL, M., MARANDA, B., MITCHELL, G., NADEAU, A., D'ANJOU, G., VANASSE, M., SROUR, M., LAFRENIERE, R. G., DRAPEAU, P., LACAILLE, J. C., KIM, E., LEE, J. R., IGARASHI, K., HUGANIR, R. L., ROULEAU, G. A. & MICHAUD, J. L. 2011. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. *Am J Hum Genet,* 88**,** 306-16.

HAMDAN, F. F., MYERS, C. T., COSSETTE, P., LEMAY, P., SPIEGELMAN, D., LAPORTE, A. D., NASSIF, C., DIALLO, O., MONLONG, J., CADIEUX-DION, M., DOBRZENIECKA, S., MELOCHE, C., RETTERER, K., CHO, M. T., ROSENFELD, J. A., BI, W., MASSICOTTE, C., MIGUET, M., BRUNGA, L., REGAN, B. M., MO, K., TAM, C., SCHNEIDER, A., HOLLINGSWORTH, G., FITZPATRICK, D. R., DONALDSON, A., CANHAM, N., BLAIR, E., KERR, B., FRY, A. E., THOMAS, R. H., SHELAGH, J., HURST, J. A., BRITTAIN, H., BLYTH, M., LEBEL, R. R., GERKES, E. H., DAVIS-KEPPEN, L., STEIN, Q., CHUNG, W. K., DORISON, S. J., BENKE, P. J., FASSI, E., CORSTEN-JANSSEN, N., KAMSTEEG, E. J., MAU-THEM, F. T., BRUEL, A. L., VERLOES, A., OUNAP, K., WOJCIK, M. H., ALBERT, D. V. F., VENKATESWARAN, S., WARE, T., JONES, D., LIU, Y. C., MOHAMMAD, S. S., BIZARGITY, P., BACINO, C. A., LEUZZI, V., MARTINELLI, S., DALLAPICCOLA, B., TARTAGLIA, M., BLUMKIN, L., WIERENGA, K. J., PURCARIN, G., O'BYRNE, J. J., STOCKLER, S., LEHMAN, A., KEREN, B., NOUGUES, M. C., MIGNOT, C., AUVIN, S., NAVA, C., HIATT, S. M., BEBIN, M., SHAO, Y., SCAGLIA, F., LALANI, S. R., FRYE, R. E., JARJOUR, I. T., JACQUES, S., BOUCHER, R. M., RIOU, E., SROUR, M., CARMANT, L., LORTIE, A., MAJOR, P., DIADORI, P., DUBEAU, F., D'ANJOU, G., BOURQUE, G., BERKOVIC, S. F., SADLEIR, L. G., CAMPEAU, P. M., KIBAR, Z., LAFRENIERE, R. G., GIRARD, S. L., MERCIMEK-MAHMUTOGLU, S., BOELMAN, C., ROULEAU, G. A., et al. 2017. High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies. *Am J Hum Genet,* 101**,** 664-685.

HAMDAN, F. F., SROUR, M., CAPO-CHICHI, J. M., DAOUD, H., NASSIF, C., PATRY, L., MASSICOTTE, C., AMBALAVANAN, A., SPIEGELMAN, D., DIALLO, O., HENRION, E., DIONNE-LAPORTE, A., FOUGERAT, A., PSHEZHETSKY, A. V., VENKATESWARAN, S., ROULEAU, G. A. & MICHAUD, J. L. 2014. De novo mutations in moderate or severe intellectual disability. *PLoS Genet,* 10**,** e1004772.

HAMPSON, D. R. & BLATT, G. J. 2015. Autism spectrum disorders and neuropathology of the cerebellum. *Front Neurosci,* 9**,** 420.

HAN, C., ALKHATER, R., FROUKH, T., MINASSIAN, A. G., GALATI, M., LIU, R. H., FOTOUHI, M., SOMMERFELD, J., ALFROOK, A. J., MARSHALL, C., WALKER, S., BAUER, P., SCHERER, S. W., RIESS, O., BUCHERT, R., MINASSIAN, B. A. & MCPHERSON, P. S. 2016. Epileptic Encephalopathy Caused by Mutations in the Guanine Nucleotide Exchange Factor DENND5A. *Am J Hum Genet,* 99**,** 1359-1367.

HAN, J. Y., JANG, J. H., PARK, J. & LEE, I. G. 2018. Targeted Next-Generation Sequencing of Korean Patients With Developmental Delay and/or Intellectual Disability. *Front Pediatr,* 6**,** 391.

HAN, J. Y., LEE, I. G., JANG, W., KIM, M., KIM, Y., JANG, J. H. & PARK, J. 2017. Diagnostic exome sequencing identifies a heterozygous MBD5 frameshift mutation in a family with intellectual disability and epilepsy. *Eur J Med Genet,* 60**,** 559-564.

HANSEN, J., SNOW, C., TUTTLE, E., GHONEIM, D. H., YANG, C. S., SPENCER, A., GUNTER, S. A., SMYSER, C. D., GURNETT, C. A., SHINAWI, M., DOBYNS, W. B., WHELESS, J., HALTERMAN, M. W., JANSEN, L. A., PASCHAL, B. M. & PACIORKOWSKI, A. R. 2015. De novo mutations in SIK1 cause a spectrum of developmental epilepsies. *Am J Hum Genet,* 96**,** 682-90.

HARDIES, K., CAI, Y., JARDEL, C., JANSEN, A. C., CAO, M., MAY, P., DJEMIE, T., HACHON LE CAMUS, C., KEYMOLEN, K., DECONINCK, T., BHAMBHANI, V., LONG, C., SAJAN, S. A., HELBIG, K. L., SULS, A., BALLING, R., HELBIG, I., DE JONGHE, P., DEPIENNE, C., DE CAMILLI, P. & WECKHUYSEN, S. 2016. Loss of SYNJ1 dual phosphatase activity leads to early onset refractory seizures and progressive neurological decline. *Brain,* 139**,** 2420-30.

HARDIES, K., DE KOVEL, C. G., WECKHUYSEN, S., ASSELBERGH, B., GEUENS, T., DECONINCK, T., AZMI, A., MAY, P., BRILSTRA, E., BECKER, F., BARISIC, N., CRAIU, D., BRAUN, K. P., LAL, D., THIELE, H., SCHUBERT, J., WEBER, Y., VAN 'T SLOT, R., NURNBERG, P., BALLING, R., TIMMERMAN, V., LERCHE, H., MAUDSLEY, S., HELBIG, I., SULS, A., KOELEMAN, B. P. & DE JONGHE, P. 2015. Recessive mutations in SLC13A5 result in a loss of citrate transport and cause neonatal epilepsy, developmental delay and teeth hypoplasia. *Brain,* 138**,** 3238-50.

HARKIN, L. A., MCMAHON, J. M., IONA, X., DIBBENS, L., PELEKANOS, J. T., ZUBERI, S. M., SADLEIR, L. G., ANDERMANN, E., GILL, D., FARRELL, K., CONNOLLY, M., STANLEY, T., HARBORD, M., ANDERMANN, F., WANG, J., BATISH, S. D., JONES, J. G., SELTZER, W. K., GARDNER, A., SUTHERLAND, G., BERKOVIC, S. F., MULLEY, J. C. & SCHEFFER, I. E. 2007. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain,* 130**,** 843-52.

HARRISON, V., CONNELL, L., HAYESMOORE, J., MCPARLAND, J., PIKE, M. G. & BLAIR, E. 2011. Compound heterozygous deletion of NRXN1 causing severe developmental delay with early onset epilepsy in two sisters. *Am J Med Genet A,* 155a**,** 2826-31.

HARVEY, K., DUGUID, I. C., ALLDRED, M. J., BEATTY, S. E., WARD, H., KEEP, N. H., LINGENFELTER, S. E., PEARCE, B. R., LUNDGREN, J., OWEN, M. J., SMART, T. G., LUSCHER, B., REES, M. I. & HARVEY, R. J. 2004. The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. *J Neurosci,* 24**,** 5816-26.

HASHIMOTO, K. 2015. Activation of sigma-1 receptor chaperone in the treatment of neuropsychiatric diseases and its clinical implication. *J Pharmacol Sci,* 127**,** 6-9.

HASHIMOTO, K., FUKUSHIMA, T., SHIMIZU, E., OKADA, S., KOMATSU, N., OKAMURA, N., KOIKE, K., KOIZUMI, H., KUMAKIRI, C., IMAI, K. & IYO, M. 2004. Possible role of D-serine in the pathophysiology of Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry,* 28**,** 385-8.

HAUSER, R. M., HENSHALL, D. C. & LUBIN, F. D. 2018. The Epigenetics of Epilepsy and Its Progression. *Neuroscientist,* 24**,** 186-200.

HAUSMAN-KEDEM, M., MENASCU, S., GREENSTEIN, Y. & FATTAL-VALEVSKI, A. 2020. Immunotherapy for GRIN2A and GRIN2D-related epileptic encephalopathy. *Epilepsy Res,* 163**,** 106325.

HEBB, D. 1949. *Organization of Behaviour*, Wiley.

HEINZEN, E. L., RADTKE, R. A., URBAN, T. J., CAVALLERI, G. L., DEPONDT, C., NEED, A. C., WALLEY, N. M., NICOLETTI, P., GE, D., CATARINO, C. B., DUNCAN, J. S., KASPERAVICIŪTE, D., TATE, S. K., CABOCLO, L. O., SANDER, J. W., CLAYTON, L., LINNEY, K. N., SHIANNA, K. V., GUMBS, C. E., SMITH, J., CRONIN, K. D., MAIA, J. M., DOHERTY, C. P., PANDOLFO, M., LEPPERT, D., MIDDLETON, L. T., GIBSON, R. A., JOHNSON, M. R., MATTHEWS, P. M., HOSFORD, D., KÄLVIÄINEN, R., ERIKSSON, K., KANTANEN, A. M., DORN, T., HANSEN, J., KRÄMER, G., STEINHOFF, B. J., WIESER, H. G., ZUMSTEG, D., ORTEGA, M., WOOD, N. W., HUXLEY-JONES, J., MIKATI, M., GALLENTINE, W. B., HUSAIN, A. M., BUCKLEY, P. G., STALLINGS, R. L., PODGOREANU, M. V., DELANTY, N., SISODIYA, S. M. & GOLDSTEIN, D. B. 2010. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet,* 86**,** 707-18.

HELBIG, K. L., FARWELL HAGMAN, K. D., SHINDE, D. N., MROSKE, C., POWIS, Z., LI, S., TANG, S. & HELBIG, I. 2016. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet Med,* 18**,** 898-905.

HERESCO-LEVY, U., DURRANT, A. R., ERMILOV, M., JAVITT, D. C., MIYA, K. & MORI, H. 2015. Clinical and electrophysiological effects of D-serine in a schizophrenia patient positive for anti-N-methyl-D-aspartate receptor antibodies. *Biol Psychiatry,* 77**,** e27-9.

HERNANDEZ, C. C. & MACDONALD, R. L. 2019. A structural look at GABA(A) receptor mutations linked to epilepsy syndromes. *Brain Res,* 1714**,** 234-247.

HERON, S. E., SMITH, K. R., BAHLO, M., NOBILI, L., KAHANA, E., LICCHETTA, L., OLIVER, K. L., MAZARIB, A., AFAWI, Z., KORCZYN, A., PLAZZI, G., PETROU, S., BERKOVIC, S. F., SCHEFFER, I. E. & DIBBENS, L. M. 2012. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet,* 44**,** 1188-90.

HERRING, B. E. & NICOLL, R. A. 2016. Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. *Annu Rev Physiol,* 78**,** 351-65.

HEYNE, H. O., SINGH, T., STAMBERGER, H., ABOU JAMRA, R., CAGLAYAN, H., CRAIU, D., DE JONGHE, P., GUERRINI, R., HELBIG, K. L., KOELEMAN, B. P. C., KOSMICKI, J. A., LINNANKIVI, T., MAY, P., MUHLE, H., MOLLER, R. S., NEUBAUER, B. A., PALOTIE, A., PENDZIWIAT, M., STRIANO, P., TANG, S., WU, S., PODURI, A., WEBER, Y. G., WECKHUYSEN, S., SISODIYA, S. M., DALY, M. J., HELBIG, I., LAL, D. & LEMKE, J. R. 2018. De novo variants in neurodevelopmental disorders with epilepsy. *Nat Genet,* 50**,** 1048-1053.

HICKS, S., WHEELER, D. A., PLON, S. E. & KIMMEL, M. 2011. Prediction of missense mutation functionality depends on both the algorithm and sequence alignment employed. *Hum Mutat,* 32**,** 661-8.

HINO-FUKUYO, N., KIKUCHI, A., ARAI-ICHINOI, N., NIIHORI, T., SATO, R., SUZUKI, T., KUDO, H., SATO, Y., NAKAYAMA, T., KAKISAKA, Y., KUBOTA, Y., KOBAYASHI, T., FUNAYAMA, R., NAKAYAMA, K., UEMATSU, M., AOKI, Y., HAGINOYA, K. & KURE, S. 2015. Genomic analysis identifies candidate pathogenic variants in 9 of 18 patients with unexplained West syndrome. *Hum Genet,* 134**,** 649-58.

HIRIART, E., GRUFFAT, H., BUISSON, M., MIKAELIAN, I., KEPPLER, S., MERESSE, P., MERCHER, T., BERNARD, O. A., SERGEANT, A. & MANET, E. 2005. Interaction of the Epstein-Barr virus mRNA export factor EB2 with human Spen proteins SHARP, OTT1, and a novel member of the family, OTT3, links Spen proteins with splicing regulation and mRNA export. *J Biol Chem,* 280**,** 36935-45.

HIRSCH, E., VALENTI, M. P., RUDOLF, G., SEEGMULLER, C., DE SAINT MARTIN, A., MAQUET, P., WIOLAND, N., METZ-LUTZ, M. N., MARESCAUX, C. & ARZIMANOGLOU, A. 2006. Landau-Kleffner syndrome is not an eponymic badge of ignorance. *Epilepsy Res,* 70 Suppl 1**,** S239-47.

HOLMES, G. L. 2009. The long-term effects of neonatal seizures. *Clin Perinatol,* 36**,** 901-14, vii-viii.

HOLMES, G. L. & RIVIELLO, J. J. 2001. Treatment of Childhood Idiopathic Language Deterioration with Valproate. *Epilepsy Behav,* 2**,** 272-276.

HONG, E. H., KIM, J. Y., KIM, J. H., LIM, D. S., KIM, M. & KIM, J. Y. 2018. BIG2-ARF1-RhoA-mDia1 Signaling Regulates Dendritic Golgi Polarization in Hippocampal Neurons. *Mol Neurobiol,* 55**,** 7701-7716.

HONG, S. E., SHUGART, Y. Y., HUANG, D. T., SHAHWAN, S. A., GRANT, P. E., HOURIHANE, J. O., MARTIN, N. D. & WALSH, C. A. 2000. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet,* 26**,** 93-6.

HORIO, M., MORI, H. & HASHIMOTO, K. 2013. Is D-cycloserine a prodrug for D-serine in the brain? *Biol Psychiatry,* 73**,** e33-4.

HOUDAYER, C., CAUX-MONCOUTIER, V., KRIEGER, S., BARROIS, M., BONNET, F., BOURDON, V., BRONNER, M., BUISSON, M., COULET, F., GAILDRAT, P., LEFOL, C., LÉONE, M., MAZOYER, S., MULLER, D., REMENIERAS, A., RÉVILLION, F., ROULEAU, E., SOKOLOWSKA, J., VERT, J. P., LIDEREAU, R., SOUBRIER, F., SOBOL, H., SEVENET, N., BRESSAC-DE PAILLERETS, B., HARDOUIN, A., TOSI, M., SINILNIKOVA, O. M. & STOPPA-LYONNET, D. 2012. Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. *Hum Mutat,* 33**,** 1228-38.

HOWELL, K. B., KORNBERG, A. J., HARVEY, A. S., RYAN, M. M., MACKAY, M. T., FREEMAN, J. L., RODRIGUEZ CASERO, M. V., COLLINS, K. J., HAYMAN, M., MOHAMED, A., WARE, T. L., CLARK, D., BRUNO, D. L., BURGESS, T., SLATER, H., MCGILLIVRAY, G. & LEVENTER, R. J. 2013. High resolution chromosomal microarray in undiagnosed neurological disorders. *J Paediatr Child Health,* 49**,** 716-24.

HOWELL, K. B., MCMAHON, J. M., CARVILL, G. L., TAMBUNAN, D., MACKAY, M. T., RODRIGUEZ-CASERO, V., WEBSTER, R., CLARK, D., FREEMAN, J. L., CALVERT, S., OLSON, H. E., MANDELSTAM, S., PODURI, A., MEFFORD, H. C., HARVEY, A. S. & SCHEFFER, I. E. 2015. SCN2A encephalopathy: A major cause of epilepsy of infancy with migrating focal seizures. *Neurology,* 85**,** 958-66.

HUANG, X., ZHANG, J., LIU, J. & ZHANG, X. 2019. Association study of the PDE4D gene and obsessive-compulsive disorder in a Chinese Han population. *Psychiatr Genet,* 29**,** 226-231.

HUGHES, J. R. 2011. A review of the relationships between Landau-Kleffner syndrome, electrical status epilepticus during sleep, and continuous spike-waves during sleep. *Epilepsy Behav,* 20**,** 247-53.

HUISMAN, S., MULDER, P. A., REDEKER, E., BADER, I., BISGAARD, A. M., BROOKS, A., CEREDA, A., CINCA, C., CLARK, D., CORMIER-DAIRE, V., DEARDORFF, M. A., DIDERICH, K., ELTING, M., VAN ESSEN, A., FITZPATRICK, D., GERVASINI, C., GILLESSEN-KAESBACH, G. & GIRISHA, K. M. 2017. Phenotypes and genotypes in individuals with SMC1A variants. 173**,** 2108-2125.

HUMPHREY, A., MACLEAN, C., PLOUBIDIS, G. B., GRANADER, Y., CLIFFORD, M., HASLOP, M., NEVILLE, B. G., YATES, J. R. & BOLTON, P. F. 2014. Intellectual development before and after the onset of infantile spasms: a controlled prospective longitudinal study in tuberous sclerosis. *Epilepsia,* 55**,** 108-16.

HUNDALLAH, K., ALENIZI, A., ALHASHEM, A. & TABARKI, B. 2016. Severe early-onset epileptic encephalopathy due to mutations in the KCNA2 gene: Expansion of the genotypic and phenotypic spectrum. *Eur J Paediatr Neurol,* 20**,** 657-60.

HUNT, D., LEVENTER, R. J., SIMONS, C., TAFT, R., SWOBODA, K. J., GAWNE-CAIN, M., MAGEE, A. C., TURNPENNY, P. D. & BARALLE, D. 2014. Whole exome sequencing in family trios reveals de novo mutations in PURA as a cause of severe neurodevelopmental delay and learning disability. *J Med Genet,* 51**,** 806-13.

IFFLAND, P. H., 2ND, BAYBIS, M., BARNES, A. E., LEVENTER, R. J., LOCKHART, P. J. & CRINO, P. B. 2018. DEPDC5 and NPRL3 modulate cell size, filopodial outgrowth, and localization of mTOR in neural progenitor cells and neurons. *Neurobiol Dis,* 114**,** 184-193.

INOKI, K., LI, Y., ZHU, T., WU, J. & GUAN, K. L. 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol,* 4**,** 648-57.

IOSSIFOV, I., O'ROAK, B. J., SANDERS, S. J., RONEMUS, M., KRUMM, N., LEVY, D., STESSMAN, H. A., WITHERSPOON, K. T., VIVES, L., PATTERSON, K. E., SMITH, J. D., PAEPER, B., NICKERSON, D. A., DEA, J., DONG, S., GONZALEZ, L. E., MANDELL, J. D., MANE, S. M., MURTHA, M. T., SULLIVAN, C. A., WALKER, M. F., WAQAR, Z., WEI, L., WILLSEY, A. J., YAMROM, B., LEE, Y. H., GRABOWSKA, E., DALKIC, E., WANG, Z., MARKS, S., ANDREWS, P., LEOTTA, A., KENDALL, J., HAKKER, I., ROSENBAUM, J., MA, B., RODGERS, L., TROGE, J., NARZISI, G., YOON, S., SCHATZ, M. C., YE, K., MCCOMBIE, W. R., SHENDURE, J., EICHLER, E. E., STATE, M. W. & WIGLER, M. 2014. The contribution of de novo coding mutations to autism spectrum disorder. *Nature,* 515**,** 216-21.

IVANOVA, D., DIRKS, A. & FEJTOVA, A. 2016. Bassoon and piccolo regulate ubiquitination and link presynaptic molecular dynamics with activity-regulated gene expression. *J Physiol,* 594**,** 5441-8.

IYADURAI, S. J. P. 2020. Congenital Myasthenic Syndromes. *Neurol Clin,* 38**,** 541-552.

JACKO, M., WEYN-VANHENTENRYCK, S. M., SMERDON, J. W., YAN, R., FENG, H., WILLIAMS, D. J., PAI, J., XU, K., WICHTERLE, H. & ZHANG, C. 2018. Rbfox Splicing Factors Promote Neuronal Maturation and Axon Initial Segment Assembly. *Neuron,* 97**,** 853-868.e6.

JAMAL, S. M., BASRAN, R. K., NEWTON, S., WANG, Z. & MILUNSKY, J. M. 2010. Novel de novo PCDH19 mutations in three unrelated females with epilepsy female restricted mental retardation syndrome. *Am J Med Genet A,* 152a**,** 2475-81.

JANSEN, S., KLEEFSTRA, T., WILLEMSEN, M. H., DE VRIES, P., PFUNDT, R., HEHIR-KWA, J. Y., GILISSEN, C., VELTMAN, J. A., DE VRIES, B. B. & VISSERS, L. E. 2016. De novo loss-of-function mutations in X-linked SMC1A cause severe ID and therapy-resistant epilepsy in females: expanding the phenotypic spectrum. *Clin Genet,* 90**,** 413-419.

JEANNE, M. & GOULD, D. B. 2017. Genotype-phenotype correlations in pathology caused by collagen type IV alpha 1 and 2 mutations. *Matrix Biol,* 57-58**,** 29-44.

JI, J., LEE, H., ARGIROPOULOS, B., DORRANI, N., MANN, J., MARTINEZ-AGOSTO, J. A., GOMEZ-OSPINA, N., GALLANT, N., BERNSTEIN, J. A., HUDGINS, L., SLATTERY, L., ISIDOR, B., LE CAIGNEC, C., DAVID, A., OBERSZTYN, E., WISNIOWIECKA-KOWALNIK, B., FOX, M., DEIGNAN, J. L., VILAIN, E., HENDRICKS, E., HORTON HARR, M., NOON, S. E., JACKSON, J. R., WILKENS, A., MIRZAA, G., SALAMON, N., ABRAMSON, J., ZACKAI, E. H., KRANTZ, I., INNES, A. M., NELSON, S. F., GRODY, W. W. & QUINTERO-RIVERA, F. 2015. DYRK1A haploinsufficiency causes a new recognizable syndrome with microcephaly, intellectual disability, speech impairment, and distinct facies. *Eur J Hum Genet,* 23**,** 1473-81.

JIAN, X., BOERWINKLE, E. & LIU, X. 2014. In silico tools for splicing defect prediction: a survey from the viewpoint of end users. *Genet Med,* 16**,** 497-503.

JIANG, M., LEE, C. L., SMITH, K. L. & SWANN, J. W. 1998. Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset epilepsy. *J Neurosci,* 18**,** 8356-68.

JOHANNESEN, K., MARINI, C., PFEFFER, S., MOLLER, R. S., DORN, T., NITURAD, C. E., GARDELLA, E., WEBER, Y., SONDERGARD, M., HJALGRIM, H., NIKANOROVA, M., BECKER, F., LARSEN, L. H., DAHL, H. A., MAIER, O., MEI, D., BISKUP, S., KLEIN, K. M., REIF, P. S., ROSENOW, F., ELIAS, A. F., HUDSON, C., HELBIG, K. L., SCHUBERT-BAST, S., SCORDO, M. R., CRAIU, D., DJEMIE, T., HOFFMAN-ZACHARSKA, D., CAGLAYAN, H., HELBIG, I., SERRATOSA, J., STRIANO, P., DE JONGHE, P., WECKHUYSEN, S., SULS, A., MURU, K., TALVIK, I., TALVIK, T., MUHLE, H., BORGGRAEFE, I., ROST, I., GUERRINI, R., LERCHE, H., LEMKE, J. R., RUBBOLI, G. & MALJEVIC, S. 2016. Phenotypic spectrum of GABRA1: From generalized epilepsies to severe epileptic encephalopathies. *Neurology,* 87**,** 1140-51.

JOHANNESEN, K. M., GARDELLA, E., LINNANKIVI, T., COURAGE, C., DE SAINT MARTIN, A., LEHESJOKI, A. E., MIGNOT, C., AFENJAR, A., LESCA, G., ABI-WARDE, M. T., CHELLY, J., PITON, A., MERRITT, J. L., 2ND, RODAN, L. H., TAN, W. H., BIRD, L. M., NESPECA, M., GLEESON, J. G., YOO, Y., CHOI, M., CHAE, J. H., CZAPANSKY-BEILMAN, D., REICHERT, S. C., PENDZIWIAT, M., VERHOEVEN, J. S., SCHELHAAS, H. J., DEVINSKY, O., CHRISTENSEN, J., SPECCHIO, N., TRIVISANO, M., WEBER, Y. G., NAVA, C., KEREN, B., DOUMMAR, D., SCHAEFER, E., HOPKINS, S., DUBBS, H., SHAW, J. E., PISANI, L., MYERS, C. T., TANG, S., TANG, S., PAL, D. K., MILLICHAP, J. J., CARVILL, G. L., HELBIG, K. L., MECARELLI, O. & STRIANO, P. 2018. Defining the phenotypic spectrum of SLC6A1 mutations. 59**,** 389-402.

JOHNSTONE, D. L., NGUYEN, T. T., MURAKAMI, Y., KERNOHAN, K. D., TETREAULT, M., GOLDSMITH, C., DOJA, A., WAGNER, J. D., HUANG, L., HARTLEY, T., ST-DENIS, A., LE DEIST, F., MAJEWSKI, J., BULMAN, D. E., KINOSHITA, T., DYMENT, D. A., BOYCOTT, K. M. & CAMPEAU, P. M. 2017. Compound heterozygous mutations in the gene PIGP are associated with early infantile epileptic encephalopathy. *Hum Mol Genet,* 26**,** 1706-1715.

JONES, L. K., LAM, R., MCKEE, K. K., ALEKSANDROVA, M., DOWLING, J., ALEXANDER, S. I., MALLAWAARACHCHI, A., COTTLE, D. L., SHORT, K. M., PAIS, L., MINER, J. H., MALLETT, A. J., SIMONS, C., MCCARTHY, H., YURCHENCO, P. D. & SMYTH, I. M.

2020. A mutation affecting laminin alpha 5 polymerisation gives rise to a syndromic developmental disorder. *Development,* 147.

KAGA, M., INAGAKI, M. & OHTA, R. 2014. Epidemiological study of Landau-Kleffner syndrome (LKS) in Japan. *Brain Dev,* 36**,** 284-6.

KAHLIG, K. M., RHODES, T. H., PUSCH, M., FREILINGER, T., PEREIRA-MONTEIRO, J. M., FERRARI, M. D., VAN DEN MAAGDENBERG, A. M., DICHGANS, M. & GEORGE, A. L., JR. 2008. Divergent sodium channel defects in familial hemiplegic migraine. *Proc Natl Acad Sci U S A,* 105**,** 9799-804.

KALSCHEUER, V. M., TAO, J., DONNELLY, A., HOLLWAY, G., SCHWINGER, E., KUBART, S., MENZEL, C., HOELTZENBEIN, M., TOMMERUP, N., EYRE, H., HARBORD, M., HAAN, E., SUTHERLAND, G. R., ROPERS, H. H. & GECZ, J. 2003. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. *Am J Hum Genet,* 72**,** 1401-11.

KAMIYA, K., KANEDA, M., SUGAWARA, T., MAZAKI, E., OKAMURA, N., MONTAL, M., MAKITA, N., TANAKA, M., FUKUSHIMA, K., FUJIWARA, T., INOUE, Y. & YAMAKAWA, K. 2004. A nonsense mutation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental decline. *J Neurosci,* 24**,** 2690-8.

KARACA, E., HAREL, T., PEHLIVAN, D., JHANGIANI, S. N., GAMBIN, T., COBAN AKDEMIR, Z., GONZAGA-JAUREGUI, C., ERDIN, S., BAYRAM, Y., CAMPBELL, I. M., HUNTER, J. V., ATIK, M. M., VAN ESCH, H., YUAN, B., WISZNIEWSKI, W., ISIKAY, S., YESIL, G., YUREGIR, O. O., TUG BOZDOGAN, S., ASLAN, H., AYDIN, H., TOS, T., AKSOY, A., DE VIVO, D. C., JAIN, P., GECKINLI, B. B., SEZER, O., GUL, D., DURMAZ, B., COGULU, O., OZKINAY, F., TOPCU, V., CANDAN, S., CEBI, A. H., IKBAL, M., YILMAZ GULEC, E., GEZDIRICI, A., KOPARIR, E., EKICI, F., COSKUN, S., CICEK, S., KARAER, K., KOPARIR, A., DUZ, M. B., KIRAT, E., FENERCIOGLU, E., ULUCAN, H., SEVEN, M., GURAN, T., ELCIOGLU, N., YILDIRIM, M. S., AKTAS, D., ALIKASIFOGLU, M., TURE, M., YAKUT, T., OVERTON, J. D., YUKSEL, A., OZEN, M., MUZNY, D. M., ADAMS, D. R., BOERWINKLE, E., CHUNG, W. K., GIBBS, R. A. & LUPSKI, J. R. 2015. Genes that Affect Brain Structure and Function Identified by Rare Variant Analyses of Mendelian Neurologic Disease. *Neuron,* 88**,** 499-513.

KATO, M., DAS, S., PETRAS, K., KITAMURA, K., MOROHASHI, K., ABUELO, D. N., BARR, M., BONNEAU, D., BRADY, A. F., CARPENTER, N. J., CIPERO, K. L., FRISONE, F., FUKUDA, T., GUERRINI, R., IIDA, E., ITOH, M., LEWANDA, A. F., NANBA, Y., OKA, A., PROUD, V. K., SAUGIER-VEBER, P., SCHELLEY, S. L., SELICORNI, A., SHANER, R., SILENGO, M., STEWART, F., SUGIYAMA, N., TOYAMA, J., TOUTAIN, A., VARGAS, A. L., YANAZAWA, M., ZACKAI, E. H. & DOBYNS, W. B. 2004. Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum Mutat,* 23**,** 147-59.

KATO, M., SAITSU, H., MURAKAMI, Y., KIKUCHI, K., WATANABE, S., IAI, M., MIYA, K., MATSUURA, R., TAKAYAMA, R., OHBA, C., NAKASHIMA, M., TSURUSAKI, Y., MIYAKE, N., HAMANO, S., OSAKA, H., HAYASAKA, K., KINOSHITA, T. & MATSUMOTO, N. 2014. PIGA mutations cause early-onset epileptic encephalopathies and distinctive features. *Neurology,* 82**,** 1587-96.

KATO, M., YAMAGATA, T., KUBOTA, M., ARAI, H., YAMASHITA, S., NAKAGAWA, T., FUJII, T., SUGAI, K., IMAI, K., USTER, T., CHITAYAT, D., WEISS, S., KASHII, H., KUSANO, R., MATSUMOTO, A., NAKAMURA, K., OYAZATO, Y., MAENO, M., NISHIYAMA, K., KODERA, H., NAKASHIMA, M., TSURUSAKI, Y., MIYAKE, N., SAITO, K., HAYASAKA, K., MATSUMOTO, N. & SAITSU, H. 2013. Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. *Epilepsia,* 54**,** 1282-7.

KELLERMANN, K. 1978. Recurrent aphasia with subclinical bioelectric status epilepticus during sleep. *Eur J Pediatr,* 128**,** 207-12.

KERIMOGLU, C., SAKIB, M. S., JAIN, G., BENITO, E., BURKHARDT, S., CAPECE, V., KAURANI, L., HALDER, R., AGÍS-BALBOA, R. C., STILLING, R., URBANKE, H., KRANZ, A., STEWART, A. F. & FISCHER, A. 2017. KMT2A and KMT2B Mediate Memory Function by Affecting Distinct Genomic Regions. *Cell Rep,* 20**,** 538-548.

KEVELAM, S. H., BIERAU, J., SALVARINOVA, R., AGRAWAL, S., HONZIK, T., VISSER, D., WEISS, M. M., SALOMONS, G. S., ABBINK, T. E., WAISFISZ, Q. & VAN DER KNAAP, M. S. 2015. Recessive ITPA mutations cause an early infantile encephalopathy. *Ann Neurol,* 78**,** 649-58.

KIDD, J. M., COOPER, G. M., DONAHUE, W. F., HAYDEN, H. S., SAMPAS, N., GRAVES, T., HANSEN, N., TEAGUE, B., ALKAN, C., ANTONACCI, F., HAUGEN, E., ZERR, T., YAMADA, N. A., TSANG, P., NEWMAN, T. L., TUZUN, E., CHENG, Z., EBLING, H. M., TUSNEEM, N., DAVID, R., GILLETT, W., PHELPS, K. A., WEAVER, M., SARANGA, D., BRAND, A., TAO, W., GUSTAFSON, E., MCKERNAN, K., CHEN, L., MALIG, M., SMITH, J. D., KORN, J. M., MCCARROLL, S. A., ALTSHULER, D. A., PEIFFER, D. A., DORSCHNER, M., STAMATOYANNOPOULOS, J., SCHWARTZ, D., NICKERSON, D. A., MULLIKIN, J. C., WILSON, R. K., BRUHN, L., OLSON, M. V., KAUL, R., SMITH, D. R. & EICHLER, E. E. 2008. Mapping and sequencing of structural variation from eight human genomes. *Nature,* 453**,** 56-64.

KILSTRUP-NIELSEN, C., RUSCONI, L., LA MONTANARA, P., CICERI, D., BERGO, A., BEDOGNI, F. & LANDSBERGER, N. 2012. What we know and would like to know about CDKL5 and its involvement in epileptic encephalopathy. *Neural Plast,* 2012**,** 728267.

KIM, Y. J., KHOSHKHOO, S., FRANKOWSKI, J. C., ZHU, B., ABBASI, S., LEE, S., WU, Y. E. & HUNT, R. F. 2018. Chd2 Is Necessary for Neural Circuit Development and Long-Term Memory. *Neuron,* 100**,** 1180-1193.e6.

KIM, Y. O., YANG, J. H., PARK, C., KIM, S. K., KIM, M. K., SHIN, M. G. & WOO, Y. J. 2016. A novel PIGA mutation in a family with X-linked, early-onset epileptic encephalopathy. *Brain Dev,* 38**,** 750-4.

KIRCHER, M., WITTEN, D. M., JAIN, P., O'ROAK, B. J. & COOPER, G. M. 2014. A general framework for estimating the relative pathogenicity of human genetic variants. 46**,** 310-5.

KIVITY, S., OLIVER, K. L., AFAWI, Z., DAMIANO, J. A., ARSOV, T., BAHLO, M. & BERKOVIC, S. F. 2017. SCN1A clinical spectrum includes the self-limited focal epilepsies of childhood. *Epilepsy Res,* 131**,** 9-14.

KJELDSEN, M. J., KYVIK, K. O., CHRISTENSEN, K. & FRIIS, M. L. 2001. Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs. *Epilepsy Res,* 44**,** 167-78.

KLEIN, B. J., WANG, X., CUI, G., YUAN, C., BOTUYAN, M. V., LIN, K., LU, Y., WANG, X., ZHAO, Y., BRUNS, C. J., MER, G., SHI, X. & KUTATELADZE, T. G. 2016. PHF20 Readers Link Methylation of Histone H3K4 and p53 with H4K16 Acetylation. *Cell Rep,* 17**,** 1158-1170.

KOBAYASHI, Y., TOHYAMA, J., KATO, M., AKASAKA, N., MAGARA, S., KAWASHIMA, H., OHASHI, T., SHIRAISHI, H., NAKASHIMA, M., SAITSU, H. & MATSUMOTO, N. 2016. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. *Brain Dev,* 38**,** 285-92.

KOBOW, K. & BLUMCKE, I. 2018. Epigenetics in epilepsy. *Neurosci Lett,* 667**,** 40-46.

KOCH, J., MAYR, J. A., ALHADDAD, B., RAUSCHER, C., BIERAU, J., KOVACS-NAGY, R., COENE, K. L., BADER, I., HOLZHACKER, M., PROKISCH, H., VENSELAAR, H., WEVERS, R. A., DISTELMAIER, F., POLSTER, T., LEIZ, S., BETZLER, C., STROM, T. M., SPERL, W., MEITINGER, T., WORTMANN, S. B. & HAACK, T. B. 2017. CAD mutations and uridine-responsive epileptic encephalopathy. *Brain,* 140**,** 279-286.

KODERA, H., NAKAMURA, K., OSAKA, H., MAEGAKI, Y., HAGINOYA, K., MIZUMOTO, S., KATO, M., OKAMOTO, N., IAI, M., KONDO, Y., NISHIYAMA, K., TSURUSAKI, Y., NAKASHIMA, M., MIYAKE, N., HAYASAKA, K., SUGAHARA, K., YUASA, I., WADA, Y., MATSUMOTO, N. & SAITSU, H. 2013. De novo mutations in SLC35A2 encoding a UDP-galactose transporter cause early-onset epileptic encephalopathy. *Hum Mutat,* 34**,** 1708-14.

KODERA, H., OSAKA, H., IAI, M., AIDA, N., YAMASHITA, A., TSURUSAKI, Y., NAKASHIMA, M., MIYAKE, N., SAITSU, H. & MATSUMOTO, N. 2015. Mutations in the glutaminyl-tRNA synthetase gene cause early-onset epileptic encephalopathy. *J Hum Genet,* 60**,** 97-101.

KOSSOFF, E. H., BOATMAN, D. & FREEMAN, J. M. 2003. Landau-Kleffner syndrome responsive to levetiracetam. *Epilepsy Behav,* 4**,** 571-5.

KOTHUR, K., HOLMAN, K., FARNSWORTH, E., HO, G., LORENTZOS, M., TROEDSON, C., GUPTA, S., WEBSTER, R., PROCOPIS, P. G., MENEZES, M. P., ANTONY, J., ARDERN-HOLMES, S., DALE, R. C., CHRISTODOULOU, J., GILL, D. & BENNETTS, B. 2018. Diagnostic yield of targeted massively parallel sequencing in children with epileptic encephalopathy. *Seizure,* 59**,** 132-140.

KOUSI, M., ANTTILA, V., SCHULZ, A., CALAFATO, S., JAKKULA, E., RIESCH, E., MYLLYKANGAS, L., KALIMO, H., TOPCU, M., GOKBEN, S., ALEHAN, F., LEMKE, J. R., ALBER, M., PALOTIE, A., KOPRA, O. & LEHESJOKI, A. E. 2012. Novel mutations

consolidate KCTD7 as a progressive myoclonus epilepsy gene. *J Med Genet,* 49**,** 391-9.

KRENN, M., WAGNER, M., HOTZY, C., GRAF, E., WEBER, S., BRUNET, T., LORENZ-DEPIEREUX, B., KASPRIAN, G., AULL-WATSCHINGER, S., PATARAIA, E., STOGMANN, E., ZIMPRICH, A., STROM, T. M., MEITINGER, T. & ZIMPRICH, F. 2020. Diagnostic exome sequencing in non-acquired focal epilepsies highlights a major role of GATOR1 complex genes. *J Med Genet*.

KRGOVIC, D., KOKALJ VOKAC, N., ZAGORAC, A. & GREGORIC KUMPERSCAK, H. 2018. Rare structural variants in the DOCK8 gene identified in a cohort of 439 patients with neurodevelopmental disorders. *Sci Rep,* 8**,** 9449.

KRUEGER, D. A., WILFONG, A. A., HOLLAND-BOULEY, K., ANDERSON, A. E., AGRICOLA, K., TUDOR, C., MAYS, M., LOPEZ, C. M., KIM, M. O. & FRANZ, D. N. 2013. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol,* 74**,** 679-87.

KRUEGER, D. A., WILFONG, A. A., MAYS, M., TALLEY, C. M., AGRICOLA, K., TUDOR, C., CAPAL, J., HOLLAND-BOULEY, K. & FRANZ, D. N. 2016. Long-term treatment of epilepsy with everolimus in tuberous sclerosis. *Neurology,* 87**,** 2408-2415.

KULLMANN, D. M., SCHORGE, S., WALKER, M. C. & WYKES, R. C. 2014. Gene therapy in epilepsy-is it time for clinical trials? *Nat Rev Neurol,* 10**,** 300-4.

KUNDE, S. A., RADEMACHER, N., TZSCHACH, A., WIEDERSBERG, E., ULLMANN, R., KALSCHEUER, V. M. & SHOICHET, S. A. 2013. Characterisation of de novo MAPK10/JNK3 truncation mutations associated with cognitive disorders in two unrelated patients. *Hum Genet,* 132**,** 461-71.

KURIAN, M. A., MEYER, E., VASSALLO, G., MORGAN, N. V., PRAKASH, N., PASHA, S., HAI, N. A., SHUIB, S., RAHMAN, F., WASSMER, E., CROSS, J. H., O'CALLAGHAN, F. J., OSBORNE, J. P., SCHEFFER, I. E., GISSEN, P. & MAHER, E. R. 2010. Phospholipase C beta 1 deficiency is associated with early-onset epileptic encephalopathy. *Brain,* 133**,** 2964-70.

LAGAE, L. G., SILBERSTEIN, J., GILLIS, P. L. & CASAER, P. J. 1998. Successful use of intravenous immunoglobulins in Landau-Kleffner syndrome. *Pediatr Neurol,* 18**,** 165-8.

LAI, C. S., FISHER, S. E., HURST, J. A., VARGHA-KHADEM, F. & MONACO, A. P. 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature,* 413**,** 519-23.

LAL, D., REINTHALER, E. M., ALTMÜLLER, J., TOLIAT, M. R., THIELE, H., NÜRNBERG, P., LERCHE, H., HAHN, A., MØLLER, R. S., MUHLE, H., SANDER, T., ZIMPRICH, F. & NEUBAUER, B. A. 2013. RBFOX1 and RBFOX3 mutations in rolandic epilepsy. *PLoS One,* 8**,** e73323.

LAM, W. W., MILLICHAP, J. J., SOARES, D. C., CHIN, R., MCLELLAN, A., FITZPATRICK, D. R., ELMSLIE, F., LEES, M. M., SCHAEFER, G. B. & ABBOTT, C. M. 2016. Novel de novo

EEF1A2 missense mutations causing epilepsy and intellectual disability. *Mol Genet Genomic Med,* 4**,** 465-74.

LAMBERT, M. P. & NEUHAUS, F. C. 1972. Mechanism of D-cycloserine action: alanine racemase from Escherichia coli W. *J Bacteriol,* 110**,** 978-87.

LAMBERT, N., DAUVE, C., RANZA, E., MAKRYTHANASIS, P., SANTONI, F., SLOAN-BENA, F., GIMELLI, S., BLOUIN, J. L., GUIPPONI, M., BOTTANI, A., ANTONARAKIS, S. E., KOSEL, M. M., FLUSS, J. & PAOLONI-GIACOBINO, A. 2018. Novel NEXMIF pathogenic variant in a boy with severe autistic features, intellectual disability, and epilepsy, and his mildly affected mother. *J Hum Genet,* 63**,** 847-850.

LANDAU, W. M. & KLEFFNER, F. R. 1957. Syndrome of acquired aphasia with convulsive disorder in children. *Neurology,* 7**,** 523-30.

LANDER, E. S., LINTON, L. M., BIRREN, B., NUSBAUM, C., ZODY, M. C., BALDWIN, J., DEVON, K., DEWAR, K., DOYLE, M., FITZHUGH, W., FUNKE, R., GAGE, D., HARRIS, K., HEAFORD, A., HOWLAND, J., KANN, L., LEHOCZKY, J., LEVINE, R., MCEWAN, P., MCKERNAN, K., MELDRIM, J., MESIROV, J. P., MIRANDA, C., MORRIS, W., NAYLOR, J., RAYMOND, C., ROSETTI, M., SANTOS, R., SHERIDAN, A., SOUGNEZ, C., STANGE-THOMANN, Y., STOJANOVIC, N., SUBRAMANIAN, A., WYMAN, D., ROGERS, J., SULSTON, J., AINSCOUGH, R., BECK, S., BENTLEY, D., BURTON, J., CLEE, C., CARTER, N., COULSON, A., DEADMAN, R., DELOUKAS, P., DUNHAM, A., DUNHAM, I., DURBIN, R., FRENCH, L., GRAFHAM, D., GREGORY, S., HUBBARD, T., HUMPHRAY, S., HUNT, A., JONES, M., LLOYD, C., MCMURRAY, A., MATTHEWS, L., MERCER, S., MILNE, S., MULLIKIN, J. C., MUNGALL, A., PLUMB, R., ROSS, M., SHOWNKEEN, R., SIMS, S., WATERSTON, R. H., WILSON, R. K., HILLIER, L. W., MCPHERSON, J. D., MARRA, M. A., MARDIS, E. R., FULTON, L. A., CHINWALLA, A. T., PEPIN, K. H., GISH, W. R., CHISSOE, S. L., WENDL, M. C., DELEHAUNTY, K. D., MINER, T. L., DELEHAUNTY, A., KRAMER, J. B., COOK, L. L., FULTON, R. S., JOHNSON, D. L., MINX, P. J., CLIFTON, S. W., HAWKINS, T., BRANSCOMB, E., PREDKI, P., RICHARDSON, P., WENNING, S., SLEZAK, T., DOGGETT, N., CHENG, J. F., OLSEN, A., LUCAS, S., ELKIN, C., UBERBACHER, E., FRAZIER, M., et al. 2001. Initial sequencing and analysis of the human genome. *Nature,* 409**,** 860-921.

LANG, F., PELZL, L., SCHÖLS, L., HERMANN, A., FÖLLER, M., SCHÄFFER, T. E. & STOURNARAS, C. 2017. Neurons, Erythrocytes and Beyond -The Diverse Functions of Chorein. *Neurosignals,* 25**,** 117-126.

LANORE, F., BLANCHET, C., FEJTOVA, A., PINHEIRO, P., RICHTER, K., BALSCHUN, D., GUNDELFINGER, E. & MULLE, C. 2010. Impaired development of hippocampal mossy fibre synapses in mouse mutants for the presynaptic scaffold protein Bassoon. *J Physiol,* 588**,** 2133-45.

LARSSON, P. G., BAKKE, K. A., BJØRNÆS, H., HEMINGHYT, E., RYTTER, E., BRAGER-LARSEN, L. & ERIKSSON, A. S. 2012. The effect of levetiracetam on focal nocturnal epileptiform activity during sleep--a placebo-controlled double-blind cross-over study. *Epilepsy Behav,* 24**,** 44-8.

LASKY-SU, J., NEALE, B. M., FRANKE, B., ANNEY, R. J., ZHOU, K., MALLER, J. B., VASQUEZ, A. A., CHEN, W., ASHERSON, P., BUITELAAR, J., BANASCHEWSKI, T., EBSTEIN, R., GILL, M., MIRANDA, A., MULAS, F., OADES, R. D., ROEYERS, H., ROTHENBERGER, A., SERGEANT, J., SONUGA-BARKE, E., STEINHAUSEN, H. C., TAYLOR, E., DALY, M., LAIRD, N., LANGE, C. & FARAONE, S. V. 2008. Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. *Am J Med Genet B Neuropsychiatr Genet,* 147b**,** 1345-54.

LAWRENSON, I. D., KREBS, D. L., LINOSSI, E. M., ZHANG, J. G., MCLENNAN, T. J., COLLIN, C., MCRAE, H. M., KOLESNIK, T. B., KOH, K., BRITTO, J. M., KUEH, A. J., SHEIKH, B. N., EL-SAAFIN, F., NICOLA, N. A., TAN, S. S., BABON, J. J., NICHOLSON, S. E., ALEXANDER, W. S., THOMAS, T. & VOSS, A. K. 2017. Cortical Layer Inversion and Deregulation of Reelin Signaling in the Absence of SOCS6 and SOCS7. *Cereb Cortex,* 27**,** 576-588.

LAYER, R. M., CHIANG, C., QUINLAN, A. R. & HALL, I. M. 2014. LUMPY: a probabilistic framework for structural variant discovery. *Genome Biol,* 15**,** R84.

LE DUC, D., GIULIVI, C., HIATT, S. M., NAPOLI, E., PANOUTSOPOULOS, A., HARLAN DE CRESCENZO, A., KOTZAERIDOU, U., SYRBE, S., ANAGNOSTOU, E., AZAGE, M., BEND, R., BEGTRUP, A., BROWN, N. J., BÜTTNER, B., CHO, M. T., COOPER, G. M., DOERING, J. H., DUBOURG, C., EVERMAN, D. B., HILDEBRAND, M. S., SANTOS, F. J. R., KELLAM, B., KELLER-RAMEY, J., LEMKE, J. R., LIU, S., NIYAZOV, D., PAYNE, K., PERSON, R., QUÉLIN, C., SCHNUR, R. E., SMITH, B. T., STROBER, J., WALKER, S., WALLIS, M., WALSH, L., YANG, S., YUEN, R. K. C., ZIEGLER, A., STICHT, H., PRIDE, M. C., OROSCO, L., MARTÍNEZ-CERDEÑO, V., SILVERMAN, J. L., CRAWLEY, J. N., SCHERER, S. W., ZARBALIS, K. S. & JAMRA, R. 2019. Pathogenic WDFY3 variants cause neurodevelopmental disorders and opposing effects on brain size. *Brain,* 142**,** 2617-2630.

LE GOFF, C. & CORMIER-DAIRE, V. 2011. The ADAMTS(L) family and human genetic disorders. *Hum Mol Genet,* 20**,** R163-7.

LE, S. V., LE, P. H. T., LE, T. K. V., KIEU HUYNH, T. T. & HANG DO, T. T. 2017. A mutation in GABRB3 associated with Dravet syndrome. 173**,** 2126-2131.

LEBRUN, N., GIURGEA, I., GOLDENBERG, A., DIEUX, A., AFENJAR, A., GHOUMID, J., DIEBOLD, B., MIETTON, L., BRIAND-SULEAU, A., BILLUART, P. & BIENVENU, T. 2018. Molecular and cellular issues of KMT2A variants involved in Wiedemann-Steiner syndrome. *Eur J Hum Genet,* 26**,** 107-116.

LEE, B. H., REIJNDERS, M. R. F., ABUBAKARE, O., TUTTLE, E., LAPE, B., MINKS, K. Q., STODGELL, C., BENNETTO, L., KWON, J., FONG, C. T., GRIPP, K. W., MARSH, E. D., SMITH, W. E., HUQ, A. M., COURY, S. A. & TAN, W. H. 2018. Expanding the neurodevelopmental phenotype of PURA syndrome. 176**,** 56-67.

LEMKE, J. R., GEIDER, K., HELBIG, K. L., HEYNE, H. O., SCHUTZ, H., HENTSCHEL, J., COURAGE, C., DEPIENNE, C., NAVA, C., HERON, D., MOLLER, R. S., HJALGRIM, H., LAL, D., NEUBAUER, B. A., NURNBERG, P., THIELE, H., KURLEMANN, G., ARNOLD,

G. L., BHAMBHANI, V., BARTHOLDI, D., PEDURUPILLAY, C. R., MISCEO, D., FRENGEN, E., STROMME, P., DLUGOS, D. J., DOHERTY, E. S., BIJLSMA, E. K., RUIVENKAMP, C. A., HOFFER, M. J., GOLDSTEIN, A., RAJAN, D. S., NARAYANAN, V., RAMSEY, K., BELNAP, N., SCHRAUWEN, I., RICHHOLT, R., KOELEMAN, B. P., SA, J., MENDONCA, C., DE KOVEL, C. G., WECKHUYSEN, S., HARDIES, K., DE JONGHE, P., DE MEIRLEIR, L., MILH, M., BADENS, C., LEBRUN, M., BUSA, T., FRANCANNET, C., PITON, A., RIESCH, E., BISKUP, S., VOGT, H., DORN, T., HELBIG, I., MICHAUD, J. L., LAUBE, B. & SYRBE, S. 2016. Delineating the GRIN1 phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. *Neurology,* 86**,** 2171-8.

LEMKE, J. R., HENDRICKX, R., GEIDER, K., LAUBE, B., SCHWAKE, M., HARVEY, R. J., JAMES, V. M., PEPLER, A., STEINER, I., HORTNAGEL, K., NEIDHARDT, J., RUF, S., WOLFF, M., BARTHOLDI, D., CARABALLO, R., PLATZER, K., SULS, A., DE JONGHE, P., BISKUP, S. & WECKHUYSEN, S. 2014. GRIN2B mutations in West syndrome and intellectual disability with focal epilepsy. *Ann Neurol,* 75**,** 147-54.

LEMKE, J. R., LAL, D., REINTHALER, E. M., STEINER, I., NOTHNAGEL, M., ALBER, M., GEIDER, K., LAUBE, B., SCHWAKE, M., FINSTERWALDER, K., FRANKE, A., SCHILHABEL, M., JAHN, J. A., MUHLE, H., BOOR, R., VAN PAESSCHEN, W., CARABALLO, R., FEJERMAN, N., WECKHUYSEN, S., DE JONGHE, P., LARSEN, J., MOLLER, R. S., HJALGRIM, H., ADDIS, L., TANG, S., HUGHES, E., PAL, D. K., VERI, K., VAHER, U., TALVIK, T., DIMOVA, P., GUERRERO LOPEZ, R., SERRATOSA, J. M., LINNANKIVI, T., LEHESJOKI, A. E., RUF, S., WOLFF, M., BUERKI, S., WOHLRAB, G., KROELL, J., DATTA, A. N., FIEDLER, B., KURLEMANN, G., KLUGER, G., HAHN, A., HABERLANDT, D. E., KUTZER, C., SPERNER, J., BECKER, F., WEBER, Y. G., FEUCHT, M., STEINBOCK, H., NEOPHYTHOU, B., RONEN, G. M., GRUBER-SEDLMAYR, U., GELDNER, J., HARVEY, R. J., HOFFMANN, P., HERMS, S., ALTMULLER, J., TOLIAT, M. R., THIELE, H., NURNBERG, P., WILHELM, C., STEPHANI, U., HELBIG, I., LERCHE, H., ZIMPRICH, F., NEUBAUER, B. A., BISKUP, S. & VON SPICZAK, S. 2013. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. *Nat Genet,* 45**,** 1067-72.

LENIGER, T., KANANURA, C., HUFNAGEL, A., BERTRAND, S., BERTRAND, D. & STEINLEIN, O. K. 2003. A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy. *Epilepsia,* 44**,** 981-5.

LERMAN, P., LERMAN-SAGIE, T. & KIVITY, S. 1991. Effect of early corticosteroid therapy for Landau-Kleffner syndrome. *Dev Med Child Neurol,* 33**,** 257-60.

LESCA, G., RUDOLF, G., BRUNEAU, N., LOZOVAYA, N., LABALME, A., BOUTRY-KRYZA, N., SALMI, M., TSINTSADZE, T., ADDIS, L., MOTTE, J., WRIGHT, S., TSINTSADZE, V., MICHEL, A., DOUMMAR, D., LASCELLES, K., STRUG, L., WATERS, P., DE BELLESCIZE, J., VRIELYNCK, P., DE SAINT MARTIN, A., VILLE, D., RYVLIN, P., ARZIMANOGLOU, A., HIRSCH, E., VINCENT, A., PAL, D., BURNASHEV, N., SANLAVILLE, D. & SZEPETOWSKI, P. 2013. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet,* 45**,** 1061-6.

LESCA, G., RUDOLF, G., LABALME, A., HIRSCH, E., ARZIMANOGLOU, A., GENTON, P., MOTTE, J., DE SAINT MARTIN, A., VALENTI, M. P., BOULAY, C., DE BELLESCIZE, J.,

KEO-KOSAL, P., BOUTRY-KRYZA, N., EDERY, P., SANLAVILLE, D. & SZEPETOWSKI, P. 2012. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. *Epilepsia,* 53**,** 1526-38.

LI, D., YUAN, H., ORTIZ-GONZALEZ, X. R., MARSH, E. D., TIAN, L., MCCORMICK, E. M., KOSOBUCKI, G. J., CHEN, W., SCHULIEN, A. J., CHIAVACCI, R., TANKOVIC, A., NAASE, C., BRUECKNER, F., VON STULPNAGEL-STEINBEIS, C., HU, C., KUSUMOTO, H., HEDRICH, U. B., ELSEN, G., HORTNAGEL, K., AIZENMAN, E., LEMKE, J. R., HAKONARSON, H., TRAYNELIS, S. F. & FALK, M. J. 2016. GRIN2D Recurrent De Novo Dominant Mutation Causes a Severe Epileptic Encephalopathy Treatable with NMDA Receptor Channel Blockers. *Am J Hum Genet,* 99**,** 802-816.

LI, W. & POZZO-MILLER, L. 2019. Dysfunction of the corticostriatal pathway in autism spectrum disorders. *J Neurosci Res*.

LI, X., POSCHMANN, S., CHEN, Q., FAZELI, W., OUNDJIAN, N. J., SNOEIJEN-SCHOUWENAARS, F. M., FRICKE, O., KAMSTEEG, E. J., WILLEMSEN, M. & WANG, Q. K. 2018. De novo BK channel variant causes epilepsy by affecting voltage gating but not Ca(2+) sensitivity. *Eur J Hum Genet,* 26**,** 220-229.

LI, Y., WANG, F., WU, L., ZHU, M., HE, G., CHEN, X., SUN, F., LIU, Q., WANG, X. & ZHANG, W. 2019. Cycloserine for treatment of multidrug-resistant tuberculosis: a retrospective cohort study in China. *Infect Drug Resist,* 12**,** 721-731.

LIÉGEOIS, F., BALDEWEG, T., CONNELLY, A., GADIAN, D. G., MISHKIN, M. & VARGHA-KHADEM, F. 2003. Language fMRI abnormalities associated with FOXP2 gene mutation. *Nat Neurosci,* 6**,** 1230-7.

LIEN, E., VATEVIK, A. K., OSTERN, R., HAUKANES, B. I. & HOUGE, G. 2016. A second patient with a De Novo GABRB1 mutation and epileptic encephalopathy. *Ann Neurol,* 80**,** 311-2.

LIGUORI, L., MONTICELLI, M., ALLOCCA, M., HAY MELE, B., LUKAS, J., CUBELLIS, M. V. & ANDREOTTI, G. 2020. Pharmacological Chaperones: A Therapeutic Approach for Diseases Caused by Destabilizing Missense Mutations. *Int J Mol Sci,* 21.

LIN, C. H., CHEN, P. K., CHANG, Y. C., CHUO, L. J., CHEN, Y. S., TSAI, G. E. & LANE, H. Y. 2014. Benzoate, a D-amino acid oxidase inhibitor, for the treatment of early-phase Alzheimer disease: a randomized, double-blind, placebo-controlled trial. *Biol Psychiatry,* 75**,** 678-85.

LINDY, A. S., STOSSER, M. B., BUTLER, E., DOWNTAIN-PICKERSGILL, C., SHANMUGHAM, A., RETTERER, K., BRANDT, T., RICHARD, G. & MCKNIGHT, D. A. 2018. Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. *Epilepsia,* 59**,** 1062-1071.

LIPPONEN, A., EL-OSTA, A., KASPI, A., ZIEMANN, M., KHURANA, I., KN, H., NAVARRO-FERRANDIS, V., PUHAKKA, N., PAANANEN, J. & PITKÄNEN, A. 2018. Transcription factors Tp73, Cebpd, Pax6, and Spi1 rather than DNA methylation regulate

chronic transcriptomics changes after experimental traumatic brain injury. *Acta Neuropathol Commun,* 6**,** 17.

LISMAN, J. 2017. Glutamatergic synapses are structurally and biochemically complex because of multiple plasticity processes: long-term potentiation, long-term depression, short-term potentiation and scaling. *Philos Trans R Soc Lond B Biol Sci,* 372.

LIU, H., ARAMAKI, M., FU, Y. & FORREST, D. 2017a. Retinoid-Related Orphan Receptor β and Transcriptional Control of Neuronal Differentiation. *Curr Top Dev Biol,* 125**,** 227-255.

LIU, Q., ZHANG, P., WANG, D., GU, W. & WANG, K. 2017b. Interrogating the "unsequenceable" genomic trinucleotide repeat disorders by long-read sequencing. *Genome Med,* 9**,** 65.

LIU, X., OU, S., XU, T., LIU, S., YUAN, J., HUANG, H., QIN, L., YANG, H., CHEN, L., TAN, X. & CHEN, Y. 2016. New differentially expressed genes and differential DNA methylation underlying refractory epilepsy. *Oncotarget,* 7**,** 87402-87416.

LOPES, F., BARBOSA, M., AMEUR, A., SOARES, G., DE SA, J., DIAS, A. I., OLIVEIRA, G., CABRAL, P., TEMUDO, T., CALADO, E., CRUZ, I. F., VIEIRA, J. P., OLIVEIRA, R., ESTEVES, S., SAUER, S., JONASSON, I., SYVANEN, A. C., GYLLENSTEN, U., PINTO, D. & MACIEL, P. 2016. Identification of novel genetic causes of Rett syndrome-like phenotypes. *J Med Genet,* 53**,** 190-9.

LOSSIN, C. 2009. A catalog of SCN1A variants. *Brain Dev,* 31**,** 114-30.

LUND, C., BRODTKORB, E., OYE, A. M., ROSBY, O. & SELMER, K. K. 2014. CHD2 mutations in Lennox-Gastaut syndrome. *Epilepsy Behav,* 33**,** 18-21.

MA, M., ADAMS, H. R., SELTZER, L. E., DOBYNS, W. B. & PACIORKOWSKI, A. R. 2016. Phenotype Differentiation of FOXG1 and MECP2 Disorders: A New Method for Characterization of Developmental Encephalopathies. *J Pediatr,* 178**,** 233-240.e10.

MADEO, M., STEWART, M., SUN, Y., SAHIR, N., WIETHOFF, S., CHANDRASEKAR, I., YARROW, A., ROSENFELD, J. A., YANG, Y., CORDEIRO, D., MCCORMICK, E. M., MURARESKU, C. C., JEPPERSON, T. N., MCBETH, L. J., SEIDAHMED, M. Z., EL KHASHAB, H. Y., HAMAD, M., AZZEDINE, H., CLARK, K., CORROCHANO, S., WELLS, S., ELTING, M. W., WEISS, M. M., BURN, S., MYERS, A., LANDSVERK, M., CROTWELL, P. L., WAISFISZ, Q., WOLF, N. I., NOLAN, P. M., PADILLA-LOPEZ, S., HOULDEN, H., LIFTON, R., MANE, S., SINGH, B. B., FALK, M. J., MERCIMEK-MAHMUTOGLU, S., BILGUVAR, K., SALIH, M. A., ACEVEDO-AROZENA, A. & KRUER, M. C. 2016. Loss-of-Function Mutations in FRRS1L Lead to an Epileptic-Dyskinetic Encephalopathy. *Am J Hum Genet,* 98**,** 1249-1255.

MALJEVIC, S., VEJZOVIC, S., BERNHARD, M. K., BERTSCHE, A., WEISE, S., DÖCKER, M., LERCHE, H., LEMKE, J. R., MERKENSCHLAGER, A. & SYRBE, S. 2016. Novel KCNQ3 Mutation in a Large Family with Benign Familial Neonatal Epilepsy: A Rare Cause of Neonatal Seizures. *Mol Syndromol,* 7**,** 189-196.

MANTOVANI, J. F. & LANDAU, W. M. 1980. Acquired aphasia with convulsive disorder: course and prognosis. *Neurology,* 30**,** 524-9.

MANVILLE, R. W. & ABBOTT, G. W. 2019. Teamwork: Ion channels and transporters join forces in the brain. *Neuropharmacology,* 161**,** 107601.

MARESCAUX, C., HIRSCH, E., FINCK, S., MAQUET, P., SCHLUMBERGER, E., SELLAL, F., METZ-LUTZ, M. N., ALEMBIK, Y., SALMON, E. & FRANCK, G. 1990. Landau-Kleffner syndrome: a pharmacologic study of five cases. *Epilepsia,* 31**,** 768-77.

MARQUET, V., BOURTHOUMIEU, S., DOBRESCU, A., LAROCHE-RAYNAUD, C. & YARDIN, C. 2017. Familial 1p36.3 microduplication resulting from a 1p-9q non-reciprocal translocation. *Eur J Med Genet,* 60**,** 583-588.

MARTINEZ, J. R., DHAWAN, A. & FARACH-CARSON, M. C. 2018. Modular Proteoglycan Perlecan/HSPG2: Mutations, Phenotypes, and Functions. *Genes (Basel),* 9.

MARTINEZ-GALAN, J. R., VERDEJO, A. & CAMINOS, E. 2018. TRPC1 Channels Are Expressed in Pyramidal Neurons and in a Subset of Somatostatin Interneurons in the Rat Neocortex. *Front Neuroanat,* 12**,** 15.

MARWICK, K., SKEHEL, P., HARDINGHAM, G. & WYLLIE, D. 2015. Effect of a GRIN2A de novo mutation associated with epilepsy and intellectual disability on NMDA receptor currents and Mg(2+) block in cultured primary cortical neurons. *Lancet,* 385 Suppl 1**,** S65.

MARZIN, P., MIGNOT, C., DORISON, N., DUFOUR, L., VILLE, D., KAMINSKA, A., PANAGIOTAKAKI, E., DIENPENDAELE, A. S., PENNIELLO, M. J., NOUGUES, M. C., KEREN, B., DEPIENNE, C., NAVA, C., MILH, M., VILLARD, L., RICHELME, C., RIVIER, C., WHALEN, S., HERON, D., LESCA, G. & DOUMMAR, D. 2018. Early-onset encephalopathy with paroxysmal movement disorders and epileptic seizures without hemiplegic attacks: About three children with novel ATP1A3 mutations. *Brain Dev*.

MASNADA, S., HEDRICH, U. B. S., GARDELLA, E., SCHUBERT, J., KAIWAR, C., KLEE, E. W., LANPHER, B. C., GAVRILOVA, R. H., SYNOFZIK, M., BAST, T., GORMAN, K., KING, M. D., ALLEN, N. M., CONROY, J., BEN ZEEV, B., TZADOK, M., KORFF, C., DUBOIS, F., RAMSEY, K., NARAYANAN, V., SERRATOSA, J. M., GIRALDEZ, B. G., HELBIG, I., MARSH, E., O'BRIEN, M., BERGQVIST, C. A., BINELLI, A., PORTER, B., ZAEYEN, E., HOROVITZ, D. D., WOLFF, M., MARJANOVIC, D., CAGLAYAN, H. S., ARSLAN, M., PENA, S. D. J., SISODIYA, S. M., BALESTRINI, S., SYRBE, S., VEGGIOTTI, P., LEMKE, J. R., MOLLER, R. S., LERCHE, H. & RUBBOLI, G. 2017. Clinical spectrum and genotype-phenotype associations of KCNA2-related encephalopathies. *Brain,* 140**,** 2337-2354.

MATHE, E., OLIVIER, M., KATO, S., ISHIOKA, C., HAINAUT, P. & TAVTIGIAN, S. V. 2006. Computational approaches for predicting the biological effect of p53 missense mutations: a comparison of three sequence analysis based methods. *Nucleic Acids Res,* 34**,** 1317-25.

MAURICE, T., SU, T. P., PARISH, D. W., NABESHIMA, T. & PRIVAT, A. 1994. PRE-084, a sigma selective PCP derivative, attenuates MK-801-induced impairment of learning in mice. *Pharmacol Biochem Behav,* 49**,** 859-69.

MCDONALD, T. J. W. & CERVENKA, M. C. 2020. Ketogenic Diet Therapies for Seizures and Status Epilepticus. *Semin Neurol,* 40**,** 719-729.

MCKINNEY, W. & MCGREAL, D. A. 1974. An aphasic syndrome in children. *Can Med Assoc J,* 110**,** 637-9.

MCTAGUE, A., HOWELL, K. B., CROSS, J. H., KURIAN, M. A. & SCHEFFER, I. E. 2016. The genetic landscape of the epileptic encephalopathies of infancy and childhood. *Lancet Neurol,* 15**,** 304-16.

MCTAGUE, A., NAIR, U., MALHOTRA, S., MEYER, E., TRUMP, N., GAZINA, E. V., PAPANDREOU, A., NGOH, A., ACKERMANN, S., AMBEGAONKAR, G., APPLETON, R., DESURKAR, A., ELTZE, C., KNEEN, R., KUMAR, A. V., LASCELLES, K., MONTGOMERY, T., RAMESH, V., SAMANTA, R., SCOTT, R. H., TAN, J., WHITEHOUSE, W., PODURI, A., SCHEFFER, I. E., CHONG, W. K. K., CROSS, J. H., TOPF, M., PETROU, S. & KURIAN, M. A. 2018. Clinical and molecular characterization of KCNT1-related severe early-onset epilepsy. *Neurology,* 90**,** e55-e66.

MEDINA, M. T., SUZUKI, T., ALONSO, M. E., DURON, R. M., MARTINEZ-JUAREZ, I. E., BAILEY, J. N., BAI, D., INOUE, Y., YOSHIMURA, I., KANEKO, S., MONTOYA, M. C., OCHOA, A., PRADO, A. J., TANAKA, M., MACHADO-SALAS, J., FUJIMOTO, S., ITO, M., HAMANO, S., SUGITA, K., UEDA, Y., OSAWA, M., OGUNI, H., RUBIO-DONNADIEU, F., YAMAKAWA, K. & DELGADO-ESCUETA, A. V. 2008. Novel mutations in Myoclonin1/EFHC1 in sporadic and familial juvenile myoclonic epilepsy. *Neurology,* 70**,** 2137-44.

MEISLER, M. H., O'BRIEN, J. E. & SHARKEY, L. M. 2010. Sodium channel gene family: epilepsy mutations, gene interactions and modifier effects. *J Physiol,* 588**,** 1841-8.

MEJIAS, R., ADAMCZYK, A., ANGGONO, V., NIRANJAN, T., THOMAS, G. M., SHARMA, K., SKINNER, C., SCHWARTZ, C. E., STEVENSON, R. E., FALLIN, M. D., KAUFMANN, W., PLETNIKOV, M., VALLE, D., HUGANIR, R. L. & WANG, T. 2011. Gain-of-function glutamate receptor interacting protein 1 variants alter GluA2 recycling and surface distribution in patients with autism. *Proc Natl Acad Sci U S A,* 108**,** 4920-5.

MELDRUM, B. S., VIGOUROUX, R. A. & BRIERLEY, J. B. 1973. Systemic factors and epileptic brain damage. Prolonged seizures in paralyzed, artificially ventilated baboons. *Arch Neurol,* 29**,** 82-7.

MESTEK-BOUKHIBAR, L., CLEMENT, E., JONES, W. D., DRURY, S., OCAKA, L., GAGUNASHVILI, A., LE QUESNE STABEJ, P., BACCHELLI, C., JANI, N., RAHMAN, S., JENKINS, L., HURST, J. A., BITNER-GLINDZICZ, M., PETERS, M., BEALES, P. L. & WILLIAMS, H. J. 2018. Rapid Paediatric Sequencing (RaPS): comprehensive real-

life workflow for rapid diagnosis of critically ill children. *J Med Genet,* 55**,** 721-728.

MICELI, F., SOLDOVIERI, M. V., JOSHI, N., WECKHUYSEN, S., COOPER, E. C. & TAGLIALATELA, M. 1993. KCNQ3-Related Disorders. *In:* ADAM, M. P., ARDINGER, H. H., PAGON, R. A., WALLACE, S. E., BEAN, L. J. H., STEPHENS, K. & AMEMIYA, A. (eds.) *GeneReviews((R)).* Seattle (WA): University of Washington, Seattle

University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.

MICELI, F., STRIANO, P., SOLDOVIERI, M. V., FONTANA, A., NARDELLO, R., ROBBIANO, A., BELLINI, G., ELIA, M., ZARA, F., TAGLIALATELA, M. & MANGANO, S. 2015. A novel KCNQ3 mutation in familial epilepsy with focal seizures and intellectual disability. *Epilepsia,* 56**,** e15-20.

MICHAOOWICZ, R., JOZWIAK, S., IGNATOWICZ, R. & SZWABOWSKA-ORZESZKO, E. 1988. Landau-Kleffner syndrome--epileptic aphasia in children--possible role of toxoplasma gondii infection. *Acta Paediatr Hung,* 29**,** 337-42.

MICHAUD, J. L., LACHANCE, M., HAMDAN, F. F., CARMANT, L., LORTIE, A., DIADORI, P., MAJOR, P., MEIJER, I. A., LEMYRE, E., COSSETTE, P., MEFFORD, H. C., ROULEAU, G. A. & ROSSIGNOL, E. 2014. The genetic landscape of infantile spasms. *Hum Mol Genet,* 23**,** 4846-58.

MIGNOT, C., VON STULPNAGEL, C., NAVA, C., VILLE, D., SANLAVILLE, D., LESCA, G., RASTETTER, A., GACHET, B., MARIE, Y., KORENKE, G. C., BORGGRAEFE, I., HOFFMANN-ZACHARSKA, D., SZCZEPANIK, E., RUDZKA-DYBALA, M., YIS, U., CAGLAYAN, H., ISAPOF, A., MAREY, I., PANAGIOTAKAKI, E., KORFF, C., ROSSIER, E., RIESS, A., BECK-WOEDL, S., RAUCH, A., ZWEIER, C., HOYER, J., REIS, A., MIRONOV, M., BOBYLOVA, M., MUKHIN, K., HERNANDEZ-HERNANDEZ, L., MAHER, B., SISODIYA, S., KUHN, M., GLAESER, D., WECKHUYSEN, S., MYERS, C. T., MEFFORD, H. C., HORTNAGEL, K., BISKUP, S., LEMKE, J. R., HERON, D., KLUGER, G. & DEPIENNE, C. 2016. Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy. *J Med Genet,* 53**,** 511-22.

MIKATI, M. A., EL-BITAR, M. K., NAJJAR, M. W., RBEIZ, J. J., BARADA, W. H., NAJJAR, V. F., YAKTIN, U. & TOURJUMAN, O. 2009. A child with refractory complex partial seizures, right temporal ganglioglioma, contralateral continuous electrical status epilepticus, and a secondary Landau-Kleffner autistic syndrome. *Epilepsy Behav,* 14**,** 411-7.

MIKATI, M. A., JIANG, Y. H., CARBONI, M., SHASHI, V., PETROVSKI, S., SPILLMANN, R., MILLIGAN, C. J., LI, M., GREFE, A., MCCONKIE, A., BERKOVIC, S., SCHEFFER, I., MULLEN, S., BONNER, M., PETROU, S. & GOLDSTEIN, D. 2015. Quinidine in the treatment of KCNT1-positive epilepsies. *Ann Neurol,* 78**,** 995-9.

MIKATI, M. A. & SAAB, R. 2000. Successful use of intravenous immunoglobulin as initial monotherapy in Landau-Kleffner syndrome. *Epilepsia,* 41**,** 880-6.

MIKATI, M. A., SAAB, R., FAYAD, M. N. & CHOUEIRI, R. N. 2002. Efficacy of intravenous immunoglobulin in Landau-Kleffner syndrome. *Pediatr Neurol,* 26**,** 298-300.

MILH, M., FALACE, A., VILLENEUVE, N., VANNI, N., CACCIAGLI, P., ASSERETO, S., NABBOUT, R., BENFENATI, F., ZARA, F., CHABROL, B., VILLARD, L. & FASSIO, A. 2013. Novel compound heterozygous mutations in TBC1D24 cause familial malignant migrating partial seizures of infancy. *Hum Mutat,* 34**,** 869-72.

MILLER, I. O. & SOTERO DE MENEZES, M. A. 1993. SCN1A-Related Seizure Disorders. *In:* ADAM, M. P., ARDINGER, H. H., PAGON, R. A., WALLACE, S. E., BEAN, L. J. H., STEPHENS, K. & AMEMIYA, A. (eds.) *GeneReviews((R)).* Seattle (WA): University of Washington, Seattle

MILLIGAN, C. J., LI, M., GAZINA, E. V., HERON, S. E., NAIR, U., TRAGER, C., REID, C. A., VENKAT, A., YOUNKIN, D. P., DLUGOS, D. J., PETROVSKI, S., GOLDSTEIN, D. B., DIBBENS, L. M., SCHEFFER, I. E., BERKOVIC, S. F. & PETROU, S. 2014. KCNT1 gain of function in 2 epilepsy phenotypes is reversed by quinidine. *Ann Neurol,* 75**,** 581-90.

MINASSIAN, B. A., LEE, J. R., HERBRICK, J. A., HUIZENGA, J., SODER, S., MUNGALL, A. J., DUNHAM, I., GARDNER, R., FONG, C. Y., CARPENTER, S., JARDIM, L., SATISHCHANDRA, P., ANDERMANN, E., SNEAD, O. C., 3RD, LOPES-CENDES, I., TSUI, L. C., DELGADO-ESCUETA, A. V., ROULEAU, G. A. & SCHERER, S. W. 1998. Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. *Nat Genet,* 20**,** 171-4.

MIRZAA, G. M., PACIORKOWSKI, A. R., MARSH, E. D., BERRY-KRAVIS, E. M., MEDNE, L., ALKHATEEB, A., GRIX, A., WIRRELL, E. C., POWELL, B. R., NICKELS, K. C., BURTON, B., PARAS, A., KIM, K., CHUNG, W., DOBYNS, W. B. & DAS, S. 2013. CDKL5 and ARX mutations in males with early-onset epilepsy. *Pediatr Neurol,* 48**,** 367-77.

MIYASHITA, A., ARAI, H., ASADA, T., IMAGAWA, M., MATSUBARA, E., SHOJI, M., HIGUCHI, S., URAKAMI, K., KAKITA, A., TAKAHASHI, H., TOYABE, S., AKAZAWA, K., KANAZAWA, I., IHARA, Y. & KUWANO, R. 2007. Genetic association of CTNNA3 with late-onset Alzheimer's disease in females. *Hum Mol Genet,* 16**,** 2854-69.

MOLINARI, F., KAMINSKA, A., FIERMONTE, G., BODDAERT, N., RAAS-ROTHSCHILD, A., PLOUIN, P., PALMIERI, L., BRUNELLE, F., PALMIERI, F., DULAC, O., MUNNICH, A. & COLLEAUX, L. 2009. Mutations in the mitochondrial glutamate carrier SLC25A22 in neonatal epileptic encephalopathy with suppression bursts. *Clin Genet,* 76**,** 188-94.

MOLINARI, F., RAAS-ROTHSCHILD, A., RIO, M., FIERMONTE, G., ENCHA-RAZAVI, F., PALMIERI, L., PALMIERI, F., BEN-NERIAH, Z., KADHOM, N., VEKEMANS, M., ATTIE-BITACH, T., MUNNICH, A., RUSTIN, P. & COLLEAUX, L. 2005. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *Am J Hum Genet,* 76**,** 334-9.

MOLLER, R. S. & JOHANNESEN, K. M. 2016. Precision Medicine: SCN8A Encephalopathy Treated with Sodium Channel Blockers. *Neurotherapeutics,* 13**,** 190-1.

MOLLER, R. S., WUTTKE, T. V., HELBIG, I., MARINI, C., JOHANNESEN, K. M., BRILSTRA, E. H., VAHER, U., BORGGRAEFE, I., TALVIK, I., TALVIK, T., KLUGER, G., FRANCOIS, L. L., LESCA, G., DE BELLESCIZE, J., BLICHFELDT, S., CHATRON, N., HOLERT, N., JACOBS, J., SWINKELS, M., BETZLER, C., SYRBE, S., NIKANOROVA, M., MYERS, C. T., LARSEN, L. H., VEJZOVIC, S., PENDZIWIAT, M., VON SPICZAK, S., HOPKINS, S., DUBBS, H., MANG, Y., MUKHIN, K., HOLTHAUSEN, H., VAN GASSEN, K. L., DAHL, H. A., TOMMERUP, N., MEFFORD, H. C., RUBBOLI, G., GUERRINI, R., LEMKE, J. R., LERCHE, H., MUHLE, H. & MALJEVIC, S. 2017. Mutations in GABRB3: From febrile seizures to epileptic encephalopathies. *Neurology,* 88**,** 483-492.

MORALES-LÁZARO, S. L., GONZÁLEZ-RAMÍREZ, R. & ROSENBAUM, T. 2019. Molecular Interplay Between the Sigma-1 Receptor, Steroids, and Ion Channels. *Front Pharmacol,* 10**,** 419.

MORESCO, L., BRUSCHETTINI, M., CALEVO, M. G. & SIRI, L. 2020. Pharmacological treatment for continuous spike-wave during slow wave sleep syndrome and Landau-Kleffner Syndrome. *Cochrane Database Syst Rev,* 11**,** Cd013132.

MORGAN, A., FISHER, S. E., SCHEFFER, I. & HILDEBRAND, M. 1993. FOXP2-Related Speech and Language Disorders. *In:* ADAM, M. P., ARDINGER, H. H., PAGON, R. A., WALLACE, S. E., BEAN, L. J. H., STEPHENS, K. & AMEMIYA, A. (eds.) *GeneReviews(®).* Seattle (WA): University of Washington, Seattle

MORRELL, F., WHISLER, W. W., SMITH, M. C., HOEPPNER, T. J., DE TOLEDO-MORRELL, L., PIERRE-LOUIS, S. J., KANNER, A. M., BUELOW, J. M., RISTANOVIC, R., BERGEN, D. & ET AL. 1995. Landau-Kleffner syndrome. Treatment with subpial intracortical transection. *Brain,* 118 (Pt 6)**,** 1529-46.

MORROW, E. M., YOO, S. Y., FLAVELL, S. W., KIM, T. K., LIN, Y., HILL, R. S., MUKADDES, N. M., BALKHY, S., GASCON, G., HASHMI, A., AL-SAAD, S., WARE, J., JOSEPH, R. M., GREENBLATT, R., GLEASON, D., ERTELT, J. A., APSE, K. A., BODELL, A., PARTLOW, J. N., BARRY, B., YAO, H., MARKIANOS, K., FERLAND, R. J., GREENBERG, M. E. & WALSH, C. A. 2008. Identifying autism loci and genes by tracing recent shared ancestry. *Science,* 321**,** 218-23.

MULDER, P. A., HUISMAN, S., LANDLUST, A. M., MOSS, J., PIENING, S., HENNEKAM, R. C. & VAN BALKOM, I. D. C. 2019. Development, behaviour and autism in individuals with SMC1A variants. *J Child Psychol Psychiatry,* 60**,** 305-313.

MULLEGAMA, S. V. & ELSEA, S. H. 2016. Clinical and Molecular Aspects of MBD5-Associated Neurodevelopmental Disorder (MAND). *Eur J Hum Genet,* 24**,** 1235-43.

MULLEN, S. A. & BERKOVIC, S. F. 2018. Genetic generalized epilepsies. 59**,** 1148-1153.

MULLEN, S. A., CARNEY, P. W., ROTEN, A., CHING, M., LIGHTFOOT, P. A., CHURILOV, L., NAIR, U., LI, M., BERKOVIC, S. F., PETROU, S. & SCHEFFER, I. E. 2018. Precision therapy for epilepsy due to KCNT1 mutations: A randomized trial of oral quinidine. *Neurology,* 90**,** e67-e72.

MUONA, M., BERKOVIC, S. F., DIBBENS, L. M., OLIVER, K. L., MALJEVIC, S., BAYLY, M. A., JOENSUU, T., CANAFOGLIA, L., FRANCESCHETTI, S., MICHELUCCI, R., MARKKINEN, S., HERON, S. E., HILDEBRAND, M. S., ANDERMANN, E., ANDERMANN, F., GAMBARDELLA, A., TINUPER, P., LICCHETTA, L., SCHEFFER, I. E., CRISCUOLO, C., FILLA, A., FERLAZZO, E. & AHMAD, J. 2015. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. 47**,** 39-46.

MUONA, M., FUKATA, Y., ANTTONEN, A. K., LAARI, A., PALOTIE, A., PIHKO, H., LONNQVIST, T., VALANNE, L., SOMER, M., FUKATA, M. & LEHESJOKI, A. E. 2016. Dysfunctional ADAM22 implicated in progressive encephalopathy with cortical atrophy and epilepsy. *Neurol Genet,* 2**,** e46.

MUSIO, A. 2020. The multiple facets of the SMC1A gene. *Gene,* 743**,** 144612.

MUTOH, H., KATO, M., AKITA, T., SHIBATA, T., WAKAMOTO, H., IKEDA, H., KITAURA, H., AOTO, K., NAKASHIMA, M., WANG, T., OHBA, C., MIYATAKE, S., MIYAKE, N., KAKITA, A., MIYAKE, K., FUKUDA, A., MATSUMOTO, N. & SAITSU, H. 2018. Biallelic Variants in CNPY3, Encoding an Endoplasmic Reticulum Chaperone, Cause Early-Onset Epileptic Encephalopathy. *Am J Hum Genet*.

MYERS, C. T. & MEFFORD, H. C. 2015. Advancing epilepsy genetics in the genomic era. *Genome Med,* 7**,** 91.

MYERS, K. A., NASIOULAS, S., BOYS, A., MCMAHON, J. M., SLATER, H., LOCKHART, P., SART, D. D. & SCHEFFER, I. E. 2018. ADGRV1 is implicated in myoclonic epilepsy. *Epilepsia,* 59**,** 381-388.

NAKAMURA, K., IKEUCHI, T., NARA, K., RHODES, C. S., ZHANG, P., CHIBA, Y., KAZUNO, S., MIURA, Y., AGO, T., ARIKAWA-HIRASAWA, E., MUKOUYAMA, Y. S. & YAMADA, Y. 2019. Perlecan regulates pericyte dynamics in the maintenance and repair of the blood-brain barrier. *J Cell Biol,* 218**,** 3506-3525.

NAKAMURA, K., KODERA, H., AKITA, T., SHIINA, M., KATO, M., HOSHINO, H., TERASHIMA, H., OSAKA, H., NAKAMURA, S., TOHYAMA, J., KUMADA, T., FURUKAWA, T., IWATA, S., SHIIHARA, T., KUBOTA, M., MIYATAKE, S., KOSHIMIZU, E., NISHIYAMA, K., NAKASHIMA, M., TSURUSAKI, Y., MIYAKE, N., HAYASAKA, K., OGATA, K., FUKUDA, A., MATSUMOTO, N. & SAITSU, H. 2013. De Novo mutations in GNAO1, encoding a Galphao subunit of heterotrimeric G proteins, cause epileptic encephalopathy. *Am J Hum Genet,* 93**,** 496-505.

NAKASHIMA, M., KATO, M., AOTO, K., SHIINA, M., BELAL, H., MUKAIDA, S., KUMADA, S., SATO, A., ZEREM, A., LERMAN-SAGIE, T., LEV, D., LEONG, H. Y., TSURUSAKI, Y., MIZUGUCHI, T., MIYATAKE, S., MIYAKE, N., OGATA, K., SAITSU, H. & MATSUMOTO, N. 2018. De novo hotspot variants in CYFIP2 cause early-onset epileptic encephalopathy. *Ann Neurol,* 83**,** 794-806.

NAKASHIMA, M., TAKANO, K., OSAKA, H., AIDA, N., TSURUSAKI, Y., MIYAKE, N., SAITSU, H. & MATSUMOTO, N. 2014. Causative novel PNKP mutations and concomitant PCDH15 mutations in a patient with microcephaly with early-onset seizures and developmental delay syndrome and hearing loss. *J Hum Genet,* 59**,** 471-4.

NAMEKATA, K., GUO, X., KIMURA, A., ARAI, N., HARADA, C. & HARADA, T. 2019. DOCK8 is expressed in microglia, and it regulates microglial activity during neurodegeneration in murine disease models. *J Biol Chem,* 294**,** 13421-13433.

NAPOLI, E., SONG, G. & PANOUTSOPOULOS, A. 2018a. Beyond autophagy: a novel role for autism-linked Wdfy3 in brain mitophagy. 8**,** 11348.

NAPOLI, E., SONG, G., PANOUTSOPOULOS, A., RIYADH, M. A., KAUSHIK, G., HALMAI, J., LEVENSON, R., ZARBALIS, K. S. & GIULIVI, C. 2018b. Beyond autophagy: a novel role for autism-linked Wdfy3 in brain mitophagy. *Sci Rep,* 8**,** 11348.

NAVA, C., DALLE, C., RASTETTER, A., STRIANO, P. & DE KOVEL, C. G. 2014. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. 46**,** 640-5.

NEER, E. J., SCHMIDT, C. J., NAMBUDRIPAD, R. & SMITH, T. F. 1994. The ancient regulatory-protein family of WD-repeat proteins. *Nature,* 371**,** 297-300.

NEILL, J. C., LIU, Z., SARKISIAN, M., TANDON, P., YANG, Y., STAFSTROM, C. E. & HOLMES, G. L. 1996. Recurrent seizures in immature rats: effect on auditory and visual discrimination. *Brain Res Dev Brain Res,* 95**,** 283-92.

NEVINS, A. K. & THURMOND, D. C. 2005. A direct interaction between Cdc42 and vesicle-associated membrane protein 2 regulates SNARE-dependent insulin exocytosis. *J Biol Chem,* 280**,** 1944-52.

NG, B. G., BUCKINGHAM, K. J., RAYMOND, K., KIRCHER, M., TURNER, E. H., HE, M., SMITH, J. D., EROSHKIN, A., SZYBOWSKA, M., LOSFELD, M. E., CHONG, J. X., KOZENKO, M., LI, C., PATTERSON, M. C., GILBERT, R. D., NICKERSON, D. A., SHENDURE, J., BAMSHAD, M. J. & FREEZE, H. H. 2013. Mosaicism of the UDP-galactose transporter SLC35A2 causes a congenital disorder of glycosylation. *Am J Hum Genet,* 92**,** 632-6.

NG, B. G., WOLFE, L. A., ICHIKAWA, M., MARKELLO, T., HE, M., TIFFT, C. J., GAHL, W. A. & FREEZE, H. H. 2015. Biallelic mutations in CAD, impair de novo pyrimidine biosynthesis and decrease glycosylation precursors. *Hum Mol Genet,* 24**,** 3050-7.

NG, P. C. & HENIKOFF, S. 2003. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res,* 31**,** 3812-4.

NGOH, A., BRAS, J., GUERREIRO, R., MCTAGUE, A., NG, J., MEYER, E., CHONG, W. K., BOYD, S., MACLELLAN, L., KIRKPATRICK, M. & KURIAN, M. A. 2017. TBC1D24 Mutations in a Sibship with Multifocal Polymyoclonus. *Tremor Other Hyperkinet Mov (N Y),* 7**,** 452.

NGOH, A., MCTAGUE, A., WENTZENSEN, I. M., MEYER, E., APPLEGATE, C., KOSSOFF, E. H., BATISTA, D. A., WANG, T. & KURIAN, M. A. 2014. Severe infantile epileptic encephalopathy due to mutations in PLCB1: expansion of the genotypic and phenotypic disease spectrum. *Dev Med Child Neurol,* 56**,** 1124-8.

NGOH, A. & PARKER, A. P. J. 2017. New developments in epilepsy management. *Paediatrics and Child Health,* 27**,** 281-286.

NGUYEN, T. T. M., MURAKAMI, Y., SHERIDAN, E., EHRESMANN, S., ROUSSEAU, J., ST-DENIS, A., CHAI, G., AJEAWUNG, N. F., FAIRBROTHER, L., REIMSCHISEL, T., BATEMAN, A., BERRY-KRAVIS, E., XIA, F., TARDIF, J., PARRY, D. A., LOGAN, C. V., DIGGLE, C., BENNETT, C. P., HATTINGH, L., ROSENFELD, J. A., PERRY, M. S., PARKER, M. J., LE DEIST, F., ZAKI, M. S., IGNATIUS, E., ISOHANNI, P., LONNQVIST, T., CARROLL, C. J., JOHNSON, C. A., GLEESON, J. G., KINOSHITA, T. & CAMPEAU, P. M. 2017. Mutations in GPAA1, Encoding a GPI Transamidase Complex Protein, Cause Developmental Delay, Epilepsy, Cerebellar Atrophy, and Osteopenia. *Am J Hum Genet,* 101**,** 856-865.

NI, H., JIANG, Y. W., BO, T., WANG, J. M., PAN, H. & WU, X. R. 2004. Long-term effects of neonatal seizures on subsequent N-methyl-D-aspartate receptor-1 and gamma-aminobutyric acid receptor A-alpha 1 receptor expression in hippocampus of the Wistar rat. *Neurosci Lett,* 368**,** 254-7.

NICKELS, K. & WIRRELL, E. 2008. Electrical status epilepticus in sleep. *Semin Pediatr Neurol,* 15**,** 50-60.

NICOLL, R. A. 2017. A Brief History of Long-Term Potentiation. *Neuron,* 93**,** 281-290.

NIITSU, T., FUJISAKI, M., SHIINA, A., YOSHIDA, T., HASEGAWA, T., KANAHARA, N., HASHIMOTO, T., SHIRAISHI, T., FUKAMI, G., NAKAZATO, M., SHIRAYAMA, Y., HASHIMOTO, K. & IYO, M. 2012. A randomized, double-blind, placebo-controlled trial of fluvoxamine in patients with schizophrenia: a preliminary study. *J Clin Psychopharmacol,* 32**,** 593-601.

NILIPOUR, Y., NAFISSI, S., TJUST, A. E., RAVENSCROFT, G., HOSSEIN NEJAD NEDAI, H., TAYLOR, R. L., VARASTEH, V., PEDROSA DOMELLÖF, F., ZANGI, M., TONEKABONI, S. H., OLIVÉ, M., KIISKI, K., SAGATH, L., DAVIS, M. R., LAING, N. G. & TAJSHARGHI, H. 2018. Ryanodine receptor type 3 (RYR3) as a novel gene associated with a myopathy with nemaline bodies. *Eur J Neurol,* 25**,** 841-847.

NONODA, Y., SAITO, Y., NAGAI, S., SASAKI, M., IWASAKI, T., MATSUMOTO, N., ISHII, M. & SAITSU, H. 2013. Progressive diffuse brain atrophy in West syndrome with marked hypomyelination due to SPTAN1 gene mutation. *Brain Dev,* 35**,** 280-3.

O'CALLAGHAN, F. J., LUX, A. L., DARKE, K., EDWARDS, S. W., HANCOCK, E., JOHNSON, A. L., KENNEDY, C. R., NEWTON, R. W., VERITY, C. M. & OSBORNE, J. P. 2011. The effect of lead time to treatment and of age of onset on developmental outcome at 4 years in infantile spasms: evidence from the United Kingdom Infantile Spasms Study. *Epilepsia,* 52**,** 1359-64.

OGIWARA, I., ITO, K., SAWAISHI, Y., OSAKA, H., MAZAKI, E., INOUE, I., MONTAL, M., HASHIKAWA, T., SHIKE, T., FUJIWARA, T., INOUE, Y., KANEDA, M. & YAMAKAWA, K. 2009. De novo mutations of voltage-gated sodium channel alphaII gene SCN2A in intractable epilepsies. *Neurology,* 73**,** 1046-53.

OGIWARA, I., NAKAYAMA, T., YAMAGATA, T., OHTANI, H., MAZAKI, E., TSUCHIYA, S., INOUE, Y. & YAMAKAWA, K. 2012. A homozygous mutation of voltage-gated sodium channel beta(I) gene SCN1B in a patient with Dravet syndrome. *Epilepsia,* 53**,** e200-3.

OGUNI, H., NISHIKAWA, A., SATO, Y., OTANI, Y., ITO, S., NAGATA, S., KATO, M., HAMANAKA, K., MIYATAKE, S. & MATSUMOTO, N. 2019. A missense variant of SMC1A causes periodic pharmaco-resistant cluster seizures similar to PCDH19-related epilepsy. *Epilepsy Res,* 155**,** 106149.

OH, J. Y., LIM, C. S., YOO, K. S., PARK, H., PARK, Y. S., KIM, E. G., LEE, Y. S., KAANG, B. K. & KIM, H. K. 2018. Adenomatous polyposis coli-stimulated GEF 1 (Asef1) is a negative regulator of excitatory synaptic function. *J Neurochem,* 147**,** 595-608.

OHBA, C., KATO, M., TAKAHASHI, S., LERMAN-SAGIE, T., LEV, D., TERASHIMA, H., KUBOTA, M., KAWAWAKI, H., MATSUFUJI, M., KOJIMA, Y., TATENO, A., GOLDBERG-STERN, H., STRAUSSBERG, R., MAROM, D., LESHINSKY-SILVER, E., NAKASHIMA, M., NISHIYAMA, K., TSURUSAKI, Y., MIYAKE, N., TANAKA, F., MATSUMOTO, N. & SAITSU, H. 2014. Early onset epileptic encephalopathy caused by de novo SCN8A mutations. *Epilepsia,* 55**,** 994-1000.

OHBA, C., SHIINA, M., TOHYAMA, J., HAGINOYA, K., LERMAN-SAGIE, T., OKAMOTO, N., BLUMKIN, L., LEV, D., MUKAIDA, S., NOZAKI, F., UEMATSU, M., ONUMA, A., KODERA, H., NAKASHIMA, M., TSURUSAKI, Y., MIYAKE, N., TANAKA, F., KATO, M., OGATA, K., SAITSU, H. & MATSUMOTO, N. 2015. GRIN1 mutations cause encephalopathy with infantile-onset epilepsy, and hyperkinetic and stereotyped movement disorders. *Epilepsia,* 56**,** 841-8.

OKABE, S. 2007. Molecular anatomy of the postsynaptic density. *Mol Cell Neurosci,* 34**,** 503-18.

OLSON, H. E., JEAN-MARCAIS, N., YANG, E., HERON, D., TATTON-BROWN, K., VAN DER ZWAAG, P. A., BIJLSMA, E. K., KROCK, B. L., BACKER, E., KAMSTEEG, E. J., SINNEMA, M., REIJNDERS, M. R. F., BEARDEN, D., BEGTRUP, A., TELEGRAFI, A., LUNSING, R. J., BURGLEN, L., LESCA, G., CHO, M. T., SMITH, L. A., SHEIDLEY, B. R., MOUFAWAD EL ACHKAR, C., PEARL, P. L., PODURI, A., SKRABAN, C. M., TARPINIAN, J., NESBITT, A. I., FRANSEN VAN DE PUTTE, D. E., RUIVENKAMP, C. A. L., RUMP, P., CHATRON, N., SABATIER, I., DE BELLESCIZE, J., GUIBAUD, L., SWEETSER, D. A., WAXLER, J. L., WIERENGA, K. J., DONADIEU, J., NARAYANAN, V., RAMSEY, K. M., NAVA, C., RIVIERE, J. B., VITOBELLO, A., TRAN MAU-THEM, F., PHILIPPE, C., BRUEL, A. L., DUFFOURD, Y., THOMAS, L., LELIEVELD, S. H., SCHUURS-HOEIJMAKERS, J., BRUNNER, H. G., KEREN, B., THEVENON, J., FAIVRE, L., THOMAS, G. & THAUVIN-ROBINET, C. 2018. A Recurrent De Novo PACS2 Heterozygous Missense Variant Causes Neonatal-Onset Developmental Epileptic

Encephalopathy, Facial Dysmorphism, and Cerebellar Dysgenesis. *Am J Hum Genet,* 102**,** 995-1007.

OMAR, M. H., KERRISK CAMPBELL, M., XIAO, X., ZHONG, Q., BRUNKEN, W. J., MINER, J. H., GREER, C. A. & KOLESKE, A. J. 2017. CNS Neurons Deposit Laminin α5 to Stabilize Synapses. *Cell Rep,* 21**,** 1281-1292.

OROSCO, L. A., ROSS, A. P., CATES, S. L., SCOTT, S. E., WU, D., SOHN, J., PLEASURE, D., PLEASURE, S. J., ADAMOPOULOS, I. E. & ZARBALIS, K. S. 2014. Loss of Wdfy3 in mice alters cerebral cortical neurogenesis reflecting aspects of the autism pathology. *Nat Commun,* 5**,** 4692.

OTERO, E., CORDOVA, S., DIAZ, F., GARCIA-TERUEL, I. & DEL BRUTTO, O. H. 1989. Acquired epileptic aphasia (the Landau-Kleffner syndrome) due to neurocysticercosis. *Epilepsia,* 30**,** 569-72.

OVERWATER, I. E., RIETMAN, A. B., BINDELS-DE HEUS, K., LOOMAN, C. W., RIZOPOULOS, D., SIBINDI, T. M., CHERIAN, P. J., JANSEN, F. E., MOLL, H. A., ELGERSMA, Y. & DE WIT, M. C. 2016. Sirolimus for epilepsy in children with tuberous sclerosis complex: A randomized controlled trial. *Neurology,* 87**,** 1011-8.

PACIORKOWSKI, A. R., TRAYLOR, R. N., ROSENFELD, J. A., HOOVER, J. M., HARRIS, C. J., WINTER, S., LACASSIE, Y., BIALER, M., LAMB, A. N., SCHULTZ, R. A., BERRY-KRAVIS, E., PORTER, B. E., FALK, M., VENKAT, A., VANZO, R. J., COHEN, J. S., FATEMI, A., DOBYNS, W. B., SHAFFER, L. G., BALLIF, B. C. & MARSH, E. D. 2013. MEF2C Haploinsufficiency features consistent hyperkinesis, variable epilepsy, and has a role in dorsal and ventral neuronal developmental pathways. *Neurogenetics,* 14**,** 99-111.

PAL, D. K., URSU, D., GAO, K., TANKOVIC, A., ZHANG, Y., KUSUMOTO, H., ZHANG, J., CHEN, W., XIANGWEI, W., SHAULSKY, G. H., HU, C., TRAYNELIS, S. F., YUAN, H. & JIANG, Y. 2017. A de novo loss-of-function GRIN2A mutation associated with childhood focal epilepsy and acquired epileptic aphasia. *Sci Rep,* 12**,** e0170818.

PALMER, E. E., JARRETT, K. E., SACHDEV, R. K., AL ZAHRANI, F., HASHEM, M. O., IBRAHIM, N., SAMPAIO, H., KANDULA, T., MACINTOSH, R., GUPTA, R., CONLON, D. M., BILLHEIMER, J. T., RADER, D. J., FUNATO, K., WALKEY, C. J., LEE, C. S., LOO, C., BRAMMAH, S., ELAKIS, G., ZHU, Y., BUCKLEY, M., KIRK, E. P., BYE, A., ALKURAYA, F. S., ROSCIOLI, T. & LAGOR, W. R. 2016. Neuronal deficiency of ARV1 causes an autosomal recessive epileptic encephalopathy. *Hum Mol Genet,* 25**,** 3042-3054.

PANJWANI, N., WILSON, M. D., ADDIS, L., CROSBIE, J., WIRRELL, E., AUVIN, S., CARABALLO, R. H., KINALI, M., MCCORMICK, D., OREN, C., TAYLOR, J., TROUNCE, J., CLARKE, T., AKMAN, C. I., KUGLER, S. L., MANDELBAUM, D. E., MCGOLDRICK, P., WOLF, S. M., ARNOLD, P., SCHACHAR, R., PAL, D. K. & STRUG, L. J. 2016. A microRNA-328 binding site in PAX6 is associated with centrotemporal spikes of rolandic epilepsy. *Ann Clin Transl Neurol,* 3**,** 512-22.

PANTE, G., THOMPSON, J., LAMBALLE, F., IWATA, T., FERBY, I., BARR, F. A., DAVIES, A. M., MAINA, F. & KLEIN, R. 2005. Mitogen-inducible gene 6 is an endogenous

inhibitor of HGF/Met-induced cell migration and neurite growth. *J Cell Biol,* 171**,** 337-48.

PAOLETTI, P. & NEYTON, J. 2007. NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol,* 7**,** 39-47.

PAPANDREOU, A., MCTAGUE, A., TRUMP, N., AMBEGAONKAR, G., NGOH, A., MEYER, E., SCOTT, R. H. & KURIAN, M. A. 2016. GABRB3 mutations: a new and emerging cause of early infantile epileptic encephalopathy. *Dev Med Child Neurol,* 58**,** 416-20.

PAPOUIN, T., LADEPECHE, L., RUEL, J., SACCHI, S., LABASQUE, M., HANINI, M., GROC, L., POLLEGIONI, L., MOTHET, J. P. & OLIET, S. H. 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell,* 150**,** 633-46.

PAPP-HERTELENDI, R., TENYI, T., HADZSIEV, K., HAU, L., BENYUS, Z. & CSABI, G. 2018. First report on the association of SCN1A mutation, childhood schizophrenia and autism spectrum disorder without epilepsy. *Psychiatry Res*.

PASCUAL-CASTROVIEJO, I., LOPEZ MARTIN, V., MARTINEZ BERMEJO, A. & PEREZ HIGUERAS, A. 1992. Is cerebral arteritis the cause of the Landau-Kleffner syndrome? Four cases in childhood with angiographic study. *Can J Neurol Sci,* 19**,** 46-52.

PATINO, G. A., CLAES, L. R., LOPEZ-SANTIAGO, L. F., SLAT, E. A., DONDETI, R. S., CHEN, C., O'MALLEY, H. A., GRAY, C. B., MIYAZAKI, H., NUKINA, N., OYAMA, F., DE JONGHE, P. & ISOM, L. L. 2009. A functional null mutation of SCN1B in a patient with Dravet syndrome. *J Neurosci,* 29**,** 10764-78.

PATRY, G., LYAGOUBI, S. & TASSINARI, C. A. 1971. Subclinical "electrical status epilepticus" induced by sleep in children. A clinical and electroencephalographic study of six cases. *Arch Neurol,* 24**,** 242-52.

PAVLIDIS, E., MØLLER, R. S., NIKANOROVA, M., KÖLMEL, M. S., STENDEVAD, P., BENICZKY, S., TASSINARI, C. A., RUBBOLI, G. & GARDELLA, E. 2019. Idiopathic encephalopathy related to status epilepticus during slow sleep (ESES) as a "pure" model of epileptic encephalopathy. An electroclinical, genetic, and follow-up study. *Epilepsy Behav,* 97**,** 244-252.

PEARL, P. L., CARRAZANA, E. J. & HOLMES, G. L. 2001. The Landau-Kleffner Syndrome. *Epilepsy Curr,* 1**,** 39-45.

PEDERICK, D. T., RICHARDS, K. L., PILTZ, S. G., KUMAR, R., MINCHEVA-TASHEVA, S., MANDELSTAM, S. A., DALE, R. C., SCHEFFER, I. E., GECZ, J., PETROU, S., HUGHES, J. N. & THOMAS, P. Q. 2018. Abnormal Cell Sorting Underlies the Unique X-Linked Inheritance of PCDH19 Epilepsy. *Neuron,* 97**,** 59-66.e5.

PENA, S. D. & COIMBRA, R. L. 2015. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in KCNA2: proposal for a new channelopathy. *Clin Genet,* 87**,** e1-3.

PENG, J., WANG, Y., HE, F., CHEN, C., WU, L. W., YANG, L. F., MA, Y. P., ZHANG, W., SHI, Z. Q., CHEN, C., XIA, K., GUO, H., YIN, F. & PANG, N. 2018. Novel West syndrome candidate genes in a Chinese cohort. *CNS Neurosci Ther,* 24**,** 1196-1206.

PENKE, B., FULOP, L., SZUCS, M. & FRECSKA, E. 2018. The Role of Sigma-1 Receptor, an Intracellular Chaperone in Neurodegenerative Diseases. *Curr Neuropharmacol,* 16**,** 97-116.

PÉREZ-PALMA, E., HELBIG, I., KLEIN, K. M., ANTTILA, V., HORN, H., REINTHALER, E. M., GORMLEY, P., GANNA, A., BYRNES, A., PERNHORST, K., TOLIAT, M. R., SAARENTAUS, E., HOWRIGAN, D. P., HOFFMAN, P., MIQUEL, J. F., DE FERRARI, G. V., NÜRNBERG, P., LERCHE, H., ZIMPRICH, F., NEUBAUER, B. A., BECKER, A. J., ROSENOW, F., PERUCCA, E., ZARA, F., WEBER, Y. G. & LAL, D. 2017. Heterogeneous contribution of microdeletions in the development of common generalised and focal epilepsies. *J Med Genet,* 54**,** 598-606.

PERNIOLA, T., MARGARI, L., BUTTIGLIONE, M., ANDREULA, C., SIMONE, I. L. & SANTOSTASI, R. 1993. A case of Landau-Kleffner syndrome secondary to inflammatory demyelinating disease. *Epilepsia,* 34**,** 551-6.

PERRAULT, I., HAMDAN, F. F., RIO, M., CAPO-CHICHI, J. M., BODDAERT, N., DECARIE, J. C., MARANDA, B., NABBOUT, R., SYLVAIN, M., LORTIE, A., ROUX, P. P., ROSSIGNOL, E., GERARD, X., BARCIA, G., BERQUIN, P., MUNNICH, A., ROULEAU, G. A., KAPLAN, J., ROZET, J. M. & MICHAUD, J. L. 2014. Mutations in DOCK7 in individuals with epileptic encephalopathy and cortical blindness. *Am J Hum Genet,* 94**,** 891-7.

PETROVSKI, S., WANG, Q., HEINZEN, E. L., ALLEN, A. S. & GOLDSTEIN, D. B. 2013. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet,* 9**,** e1003709.

PHELAN, K. D., SHWE, U. T., ABRAMOWITZ, J., WU, H., RHEE, S. W., HOWELL, M. D., GOTTSCHALL, P. E., FREICHEL, M., FLOCKERZI, V., BIRNBAUMER, L. & ZHENG, F. 2013. Canonical transient receptor channel 5 (TRPC5) and TRPC1/4 contribute to seizure and excitotoxicity by distinct cellular mechanisms. *Mol Pharmacol,* 83**,** 429-38.

PIERSON, T. M., YUAN, H., MARSH, E. D., FUENTES-FAJARDO, K., ADAMS, D. R., MARKELLO, T., GOLAS, G., SIMEONOV, D. R., HOLLOMAN, C., TANKOVIC, A., KARAMCHANDANI, M. M., SCHREIBER, J. M., MULLIKIN, J. C., TIFFT, C. J., TORO, C., BOERKOEL, C. F., TRAYNELIS, S. F. & GAHL, W. A. 2014. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. *Ann Clin Transl Neurol,* 1**,** 190-198.

PINAR, C., FONTAINE, C. J., TRIVINO-PAREDES, J., LOTTENBERG, C. P., GIL-MOHAPEL, J. & CHRISTIE, B. R. 2017. Revisiting the flip side: Long-term depression of synaptic efficacy in the hippocampus. *Neurosci Biobehav Rev,* 80**,** 394-413.

PINGGERA, A., MACKENROTH, L., RUMP, A., SCHALLNER, J., BELEGGIA, F., WOLLNIK, B. & STRIESSNIG, J. 2017. New gain-of-function mutation shows CACNA1D as

recurrently mutated gene in autism spectrum disorders and epilepsy. *Hum Mol Genet,* 26**,** 2923-2932.

PIRO, E., NARDELLO, R., GENNARO, E., FONTANA, A., TAGLIALATELA, M., DONATO MANGANO, G., CORSELLO, G. & MANGANO, S. 2019. A novel mutation in KCNQ3-related benign familial neonatal epilepsy: electroclinical features and neurodevelopmental outcome. *Epileptic Disord,* 21**,** 87-91.

PISANO, T., NUMIS, A. L., HEAVIN, S. B., WECKHUYSEN, S., ANGRIMAN, M., SULS, A., PODESTA, B., THIBERT, R. L., SHAPIRO, K. A., GUERRINI, R., SCHEFFER, I. E., MARINI, C. & CILIO, M. R. 2015. Early and effective treatment of KCNQ2 encephalopathy. *Epilepsia,* 56**,** 685-91.

PIZZINO, A., WHITEHEAD, M., SABET RASEKH, P., MURPHY, J., HELMAN, G., BLOOM, M., EVANS, S. H., MURNICK, J. G., CONRY, J., TAFT, R. J., SIMONS, C., VANDERVER, A. & ADANG, L. A. 2018. Mutations in SZT2 result in early-onset epileptic encephalopathy and leukoencephalopathy. *Am J Med Genet A,* 176**,** 1443-1448.

PLATZER, K., YUAN, H., SCHUTZ, H., WINSCHEL, A., CHEN, W., HU, C., KUSUMOTO, H., HEYNE, H. O., HELBIG, K. L., TANG, S., WILLING, M. C., TINKLE, B. T., ADAMS, D. J., DEPIENNE, C., KEREN, B., MIGNOT, C., FRENGEN, E., STROMME, P., BISKUP, S., DOCKER, D., STROM, T. M., MEFFORD, H. C., MYERS, C. T., MUIR, A. M., LACROIX, A., SADLEIR, L., SCHEFFER, I. E., BRILSTRA, E., VAN HAELST, M. M., VAN DER SMAGT, J. J., BOK, L. A., MOLLER, R. S., JENSEN, U. B., MILLICHAP, J. J., BERG, A. T., GOLDBERG, E. M., DE BIE, I., FOX, S., MAJOR, P., JONES, J. R., ZACKAI, E. H., ABOU JAMRA, R., ROLFS, A., LEVENTER, R. J., LAWSON, J. A., ROSCIOLI, T., JANSEN, F. E., RANZA, E., KORFF, C. M., LEHESJOKI, A. E., COURAGE, C., LINNANKIVI, T., SMITH, D. R., STANLEY, C., MINTZ, M., MCKNIGHT, D., DECKER, A., TAN, W. H., TARNOPOLSKY, M. A., BRADY, L. I., WOLFF, M., DONDIT, L., PEDRO, H. F., PARISOTTO, S. E., JONES, K. L., PATEL, A. D., FRANZ, D. N., VANZO, R., MARCO, E., RANELLS, J. D., DI DONATO, N., DOBYNS, W. B., LAUBE, B., TRAYNELIS, S. F. & LEMKE, J. R. 2017. GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. *J Med Genet,* 54**,** 460-470.

PODURI, A. 2014. HCN1 Gain-Of-Function Mutations - A New Cause of Epileptic Encephalopathy. *Epilepsy Curr,* 14**,** 348-9.

PODURI, A., CHOPRA, S. S., NEILAN, E. G., ELHOSARY, P. C., KURIAN, M. A., MEYER, E., BARRY, B. J., KHWAJA, O. S., SALIH, M. A., STODBERG, T., SCHEFFER, I. E., MAHER, E. R., SAHIN, M., WU, B. L., BERRY, G. T., WALSH, C. A., PICKER, J. & KOTHARE, S. V. 2012. Homozygous PLCB1 deletion associated with malignant migrating partial seizures in infancy. *Epilepsia,* 53**,** e146-50.

PODURI, A., HEINZEN, E. L., CHITSAZZADEH, V., LASORSA, F. M., ELHOSARY, P. C., LACOURSIERE, C. M., MARTIN, E., YUSKAITIS, C. J., HILL, R. S., ATABAY, K. D., BARRY, B., PARTLOW, J. N., BASHIRI, F. A., ZEIDAN, R. M., ELMALIK, S. A., KABIRAJ, M. M., KOTHARE, S., STODBERG, T., MCTAGUE, A., KURIAN, M. A., SCHEFFER, I. E., BARKOVICH, A. J., PALMIERI, F., SALIH, M. A. & WALSH, C. A. 2013. SLC25A22 is a novel gene for migrating partial seizures in infancy. *Ann Neurol,* 74**,** 873-82.

POISSON, A., CHATRON, N., LABALME, A., FOURNERET, P., VILLE, D., MATHIEU, M. L., SANLAVILLE, D., DEMILY, C. & LESCA, G. 2020. Chromatin remodeling dysfunction extends the etiological spectrum of schizophrenia: a case report. *BMC Med Genet,* 21**,** 10.

POULTON, C., OEGEMA, R., HEIJSMAN, D., HOOGEBOOM, J., SCHOT, R., STROINK, H., WILLEMSEN, M. A., VERHEIJEN, F. W., VAN DE SPEK, P., KREMER, A. & MANCINI, G. M. 2013. Progressive cerebellar atrophy and polyneuropathy: expanding the spectrum of PNKP mutations. *Neurogenetics,* 14**,** 43-51.

POWIS, Z., FARWELL HAGMAN, K. D., MROSKE, C., MCWALTER, K., COHEN, J. S., COLOMBO, R., SERRETTI, A., FATEMI, A., DAVID, K. L., REYNOLDS, J., IMMKEN, L., NAGAKURA, H., CUNNIFF, C. M., PAYNE, K., BARBARO-DIEBER, T., GRIPP, K. W., BAKER, L., STAMPER, T., ALECK, K. A., JORDAN, E. S., HERSH, J. H., BURTON, J., WENTZENSEN, I. M., GUILLEN SACOTO, M. J., WILLAERT, R., CHO, M. T., PETRIK, I., HUETHER, R. & TANG, S. 2018. Expansion and further delineation of the SETD5 phenotype leading to global developmental delay, variable dysmorphic features, and reduced penetrance. *Clin Genet,* 93**,** 752-761.

PUFFENBERGER, E. G., JINKS, R. N., SOUGNEZ, C., CIBULSKIS, K., WILLERT, R. A., ACHILLY, N. P., CASSIDY, R. P., FIORENTINI, C. J., HEIKEN, K. F., LAWRENCE, J. J., MAHONEY, M. H., MILLER, C. J., NAIR, D. T., POLITI, K. A., WORCESTER, K. N., SETTON, R. A., DIPIAZZA, R., SHERMAN, E. A., EASTMAN, J. T., FRANCKLYN, C., ROBEY-BOND, S., RIDER, N. L., GABRIEL, S., MORTON, D. H. & STRAUSS, K. A. 2012. Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One,* 7**,** e28936.

PULST, S. M. 1999. Genetic linkage analysis. *Arch Neurol,* 56**,** 667-72.

QU, J., YANG, Z. Q., ZHANG, Y., MAO, C. X., WANG, Z. B., MAO, X. Y., ZHOU, B. T., YIN, J. Y., HE, H., LONG, H. Y., GONG, J. E., XIAO, B., ZHOU, H. H. & LIU, Z. Q. 2015. Common variants of ATP1A3 but not ATP1A2 are associated with Chinese genetic generalized epilepsies. *J Neurol Sci,* 354**,** 56-62.

RAJMAN, M., METGE, F., FIORE, R., KHUDAYBERDIEV, S., AKSOY-AKSEL, A., BICKER, S., RUEDELL RESCHKE, C., RAOOF, R., BRENNAN, G. P., DELANTY, N., FARRELL, M. A., O'BRIEN, D. F., BAUER, S., NORWOOD, B., VENO, M. T., KRÜGER, M., BRAUN, T., KJEMS, J., ROSENOW, F., HENSHALL, D. C., DIETERICH, C. & SCHRATT, G. 2017. A microRNA-129-5p/Rbfox crosstalk coordinates homeostatic downscaling of excitatory synapses. *Embo j,* 36**,** 1770-1787.

RAMSER, J., ABIDI, F. E., BURCKLE, C. A., LENSKI, C., TORIELLO, H., WEN, G., LUBS, H. A., ENGERT, S., STEVENSON, R. E., MEINDL, A., SCHWARTZ, C. E. & NGUYEN, G. 2005. A unique exonic splice enhancer mutation in a family with X-linked mental retardation and epilepsy points to a novel role of the renin receptor. *Hum Mol Genet,* 14**,** 1019-27.

RAUCH, A., WIECZOREK, D., GRAF, E., WIELAND, T., ENDELE, S., SCHWARZMAYR, T., ALBRECHT, B., BARTHOLDI, D., BEYGO, J., DI DONATO, N., DUFKE, A., CREMER, K., HEMPEL, M., HORN, D., HOYER, J., JOSET, P., ROPKE, A., MOOG, U., RIESS, A.,

THIEL, C. T., TZSCHACH, A., WIESENER, A., WOHLLEBER, E., ZWEIER, C., EKICI, A. B., ZINK, A. M., RUMP, A., MEISINGER, C., GRALLERT, H., STICHT, H., SCHENCK, A., ENGELS, H., RAPPOLD, G., SCHROCK, E., WIEACKER, P., RIESS, O., MEITINGER, T., REIS, A. & STROM, T. M. 2012. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet,* 380**,** 1674-82.

REBSAM, A. & MASON, C. A. 2011. Cadherins as matchmakers. *Neuron,* 71**,** 566-8.

REDIES, C., HERTEL, N. & HÜBNER, C. A. 2012. Cadherins and neuropsychiatric disorders. *Brain Res,* 1470**,** 130-44.

REDLER, S., STROM, T. M., WIELAND, T., CREMER, K., ENGELS, H., DISTELMAIER, F., SCHAPER, J., KUCHLER, A., LEMKE, J. R., JESCHKE, S., SCHREYER, N., STICHT, H., KOCH, M., LUDECKE, H. J. & WIECZOREK, D. 2017. Variants in CPLX1 in two families with autosomal-recessive severe infantile myoclonic epilepsy and ID. *Eur J Hum Genet,* 25**,** 889-893.

REESE, M. G., EECKMAN, F. H., KULP, D. & HAUSSLER, D. 1997. Improved splice site detection in Genie. *J Comput Biol,* 4**,** 311-23.

REIJNDERS, M. R. F., JANOWSKI, R., ALVI, M., SELF, J. E., VAN ESSEN, T. J., VREEBURG, M., ROUHL, R. P. W., STEVENS, S. J. C., STEGMANN, A. P. A., SCHIEVING, J., PFUNDT, R., VAN DIJK, K., SMEETS, E., STUMPEL, C., BOK, L. A., COBBEN, J. M., ENGELEN, M., MANSOUR, S., WHITEFORD, M., CHANDLER, K. E., DOUZGOU, S., COOPER, N. S., TAN, E. C., FOO, R., LAI, A. H. M., RANKIN, J., GREEN, A., LONNQVIST, T., ISOHANNI, P., WILLIAMS, S., RUHOY, I., CARVALHO, K. S., DOWLING, J. J., LEV, D. L., STERBOVA, K., LASSUTHOVA, P., NEUPAUEROVA, J., WAUGH, J. L., KEROS, S., CLAYTON-SMITH, J., SMITHSON, S. F., BRUNNER, H. G., VAN HOECKEL, C., ANDERSON, M., CLOWES, V. E., SIU, V. M., DDD STUDY, T., SELBER, P., LEVENTER, R. J., NELLAKER, C., NIESSING, D., HUNT, D. & BARALLE, D. 2018. PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature. *J Med Genet,* 55**,** 104-113.

REINTHALER, E. M., DEJANOVIC, B., LAL, D., SEMTNER, M., MERKLER, Y., REINHOLD, A., PITTRICH, D. A., HOTZY, C., FEUCHT, M., STEINBOCK, H., GRUBER-SEDLMAYR, U., RONEN, G. M., NEOPHYTOU, B., GELDNER, J., HABERLANDT, E., MUHLE, H., IKRAM, M. A., VAN DUIJN, C. M., UITTERLINDEN, A. G., HOFMAN, A., ALTMULLER, J., KAWALIA, A., TOLIAT, M. R., NURNBERG, P., LERCHE, H., NOTHNAGEL, M., THIELE, H., SANDER, T., MEIER, J. C., SCHWARZ, G., NEUBAUER, B. A. & ZIMPRICH, F. 2015. Rare variants in gamma-aminobutyric acid type A receptor genes in rolandic epilepsy and related syndromes. *Ann Neurol,* 77**,** 972-86.

REINTHALER, E. M., LAL, D., LEBON, S., HILDEBRAND, M. S., DAHL, H. H., REGAN, B. M., FEUCHT, M., STEINBÖCK, H., NEOPHYTOU, B., RONEN, G. M., ROCHE, L., GRUBER-SEDLMAYR, U., GELDNER, J., HABERLANDT, E., HOFFMANN, P., HERMS, S., GIEGER, C., WALDENBERGER, M., FRANKE, A., WITTIG, M., SCHOCH, S., BECKER, A. J., HAHN, A., MÄNNIK, K., TOLIAT, M. R., WINTERER, G., LERCHE, H., NÜRNBERG, P., MEFFORD, H., SCHEFFER, I. E., BERKOVIC, S. F., BECKMANN, J. S., SANDER, T., JACQUEMONT, S., REYMOND, A., ZIMPRICH, F. & NEUBAUER, B. A.

2014. 16p11.2 600 kb Duplications confer risk for typical and atypical Rolandic epilepsy. *Hum Mol Genet,* 23**,** 6069-80.

REISBERG, B., DOODY, R., STOFFLER, A., SCHMITT, F., FERRIS, S. & MOBIUS, H. J. 2003. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med,* 348**,** 1333-41.

REUTLINGER, C., HELBIG, I., GAWELCZYK, B., SUBERO, J. I., TONNIES, H., MUHLE, H., FINSTERWALDER, K., VERMEER, S., PFUNDT, R., SPERNER, J., STEFANOVA, I., GILLESSEN-KAESBACH, G., VON SPICZAK, S., VAN BAALEN, A., BOOR, R., SIEBERT, R., STEPHANI, U. & CALIEBE, A. 2010. Deletions in 16p13 including GRIN2A in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. *Epilepsia,* 51**,** 1870-3.

RICCIARELLI, R. & FEDELE, E. 2018. cAMP, cGMP and Amyloid beta: Three Ideal Partners for Memory Formation. *Trends Neurosci,* 41**,** 255-266.

RICHARDS, S., AZIZ, N., BALE, S., BICK, D., DAS, S., GASTIER-FOSTER, J., GRODY, W. W., HEGDE, M., LYON, E., SPECTOR, E., VOELKERDING, K. & REHM, H. L. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. 17**,** 405-24.

RICOS, M. G., HODGSON, B. L., PIPPUCCI, T., SAIDIN, A., ONG, Y. S., HERON, S. E., LICCHETTA, L., BISULLI, F., BAYLY, M. A., HUGHES, J., BALDASSARI, S., PALOMBO, F., SANTUCCI, M., MELETTI, S., BERKOVIC, S. F., RUBBOLI, G., THOMAS, P. Q., SCHEFFER, I. E., TINUPER, P., GEOGHEGAN, J., SCHREIBER, A. W. & DIBBENS, L. M. 2016. Mutations in the mammalian target of rapamycin pathway regulators NPRL2 and NPRL3 cause focal epilepsy. *Ann Neurol,* 79**,** 120-31.

RISHER, W. C., KIM, N., KOH, S., CHOI, J. E., MITEV, P., SPENCE, E. F., PILAZ, L. J., WANG, D., FENG, G., SILVER, D. L., SODERLING, S. H., YIN, H. H. & EROGLU, C. 2018. Thrombospondin receptor α2δ-1 promotes synaptogenesis and spinogenesis via postsynaptic Rac1. *J Cell Biol,* 217**,** 3747-3765.

RIZO, J., CHEN, X. & ARAC, D. 2006. Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends Cell Biol,* 16**,** 339-50.

ROBERTSON, I. B., HORIGUCHI, M., ZILBERBERG, L., DABOVIC, B., HADJIOLOVA, K. & RIFKIN, D. B. 2015. Latent TGF-β-binding proteins. *Matrix Biol,* 47**,** 44-53.

ROBINSON, R. O., BAIRD, G., ROBINSON, G. & SIMONOFF, E. 2001. Landau-Kleffner syndrome: course and correlates with outcome. *Dev Med Child Neurol,* 43**,** 243-7.

RODRIGUEZ, I. & NIEDERMEYER, E. 1982. The aphasia-epilepsy syndrome in children: electroencephalographic aspects. *Clin Electroencephalogr,* 13**,** 23-35.

ROGERS, A., GOLUMBEK, P., CELLINI, E., DOCCINI, V., GUERRINI, R., WALLGREN-PETTERSSON, C., THURESSON, A. C. & GURNETT, C. A. 2018. De novo KCNA1

variants in the PVP motif cause infantile epileptic encephalopathy and cognitive impairment similar to recurrent KCNA2 variants. *Am J Med Genet A*.

ROLL, P., RUDOLF, G., PEREIRA, S., ROYER, B., SCHEFFER, I. E., MASSACRIER, A., VALENTI, M. P., ROECKEL-TREVISIOL, N., JAMALI, S., BECLIN, C., SEEGMULLER, C., METZ-LUTZ, M. N., LEMAINQUE, A., DELEPINE, M., CALOUSTIAN, C., DE SAINT MARTIN, A., BRUNEAU, N., DEPETRIS, D., MATTEI, M. G., FLORI, E., ROBAGLIA-SCHLUPP, A., LEVY, N., NEUBAUER, B. A., RAVID, R., MARESCAUX, C., BERKOVIC, S. F., HIRSCH, E., LATHROP, M., CAU, P. & SZEPETOWSKI, P. 2006. SRPX2 mutations in disorders of language cortex and cognition. *Hum Mol Genet,* 15**,** 1195-207.

ROLL, P., VERNES, S. C., BRUNEAU, N., CILLARIO, J., PONSOLE-LENFANT, M., MASSACRIER, A., RUDOLF, G., KHALIFE, M., HIRSCH, E., FISHER, S. E. & SZEPETOWSKI, P. 2010. Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. *Hum Mol Genet,* 19**,** 4848-60.

ROSANOFF, M. J. & OTTMAN, R. 2008. Penetrance of LGI1 mutations in autosomal dominant partial epilepsy with auditory features. *Neurology,* 71**,** 567-71.

ROSSI, P. G., PARMEGGIANI, A., POSAR, A., SCADUTO, M. C., CHIODO, S. & VATTI, G. 1999. Landau-Kleffner syndrome (LKS): long-term follow-up and links with electrical status epilepticus during sleep (ESES). *Brain Dev,* 21**,** 90-8.

RUBAIY, H. N. 2019. Treasure troves of pharmacological tools to study transient receptor potential canonical 1/4/5 channels. *Br J Pharmacol,* 176**,** 832-846.

RUBIO, M. D., JOHNSON, R., MILLER, C. A., HUGANIR, R. L. & RUMBAUGH, G. 2011. Regulation of synapse structure and function by distinct myosin II motors. *J Neurosci,* 31**,** 1448-60.

RUDOLF, G., LESCA, G., MEHRJOUY, M. M., LABALME, A., SALMI, M., BACHE, I., BRUNEAU, N., PENDZIWIAT, M., FLUSS, J., DE BELLESCIZE, J., SCHOLLY, J., MØLLER, R. S., CRAIU, D., TOMMERUP, N., VALENTI-HIRSCH, M. P., SCHLUTH-BOLARD, C., SLOAN-BÉNA, F., HELBIG, K. L., WECKHUYSEN, S., EDERY, P., COULBAUT, S., ABBAS, M., SCHEFFER, I. E., TANG, S., MYERS, C. T., STAMBERGER, H., CARVILL, G. L., SHINDE, D. N., MEFFORD, H. C., NEAGU, E., HUETHER, R., LU, H. M., DICA, A., COHEN, J. S., ILIESCU, C., POMERAN, C., RUBENSTEIN, J., HELBIG, I., SANLAVILLE, D., HIRSCH, E. & SZEPETOWSKI, P. 2016. Loss of function of the retinoid-related nuclear receptor (RORB) gene and epilepsy. *Eur J Hum Genet,* 24**,** 1761-1770.

RYAN, S. G., CHANCE, P. F., ZOU, C. H., SPINNER, N. B., GOLDEN, J. A. & SMIETANA, S. 1997. Epilepsy and mental retardation limited to females: an X-linked dominant disorder with male sparing. *Nat Genet,* 17**,** 92-5.

SADHWANI, A., SANJANA, N. E., WILLEN, J. M., CALCULATOR, S. N., BLACK, E. D., BEAN, L. J. H., LI, H. & TAN, W. H. 2018. Two Angelman families with unusually advanced neurodevelopment carry a start codon variant in the most highly expressed UBE3A isoform.

SADLEIR, L. G., DE VALLES-IBÁÑEZ, G., KING, C., COLEMAN, M., MOSSMAN, S., PATERSON, S., NGUYEN, J., BERKOVIC, S. F., MULLEN, S., BAHLO, M., HILDEBRAND, M. S., MEFFORD, H. C. & SCHEFFER, I. E. 2020. Inherited RORB pathogenic variants: Overlap of photosensitive genetic generalized and occipital lobe epilepsy. *Epilepsia,* 61**,** e23-e29.

SAFFARI, A., BRÖSSE, I., WIEMER-KRUEL, A., WILKEN, B., KREUZALER, P., HAHN, A., BERNHARD, M. K., VAN TILBURG, C. M., HOFFMANN, G. F., GORENFLO, M., HETHEY, S., KAISER, O., KÖLKER, S., WAGNER, R., WITT, O., MERKENSCHLAGER, A., MÖCKEL, A., ROSER, T., SCHLUMP, J. U., SERFLING, A., SPIEGLER, J., MILDE, T., ZIEGLER, A. & SYRBE, S. 2019. Safety and efficacy of mTOR inhibitor treatment in patients with tuberous sclerosis complex under 2 years of age - a multicenter retrospective study. *Orphanet J Rare Dis,* 14**,** 96.

SAITO, T. & ISHII, A. 2017. A de novo missense mutation in SLC12A5 found in a compound heterozygote patient with epilepsy of infancy with migrating focal seizures. 92**,** 654-658.

SAITSU, H., FUKAI, R., BEN-ZEEV, B., SAKAI, Y., MIMAKI, M., OKAMOTO, N., SUZUKI, Y., MONDEN, Y., SAITO, H., TZIPERMAN, B., TORIO, M., AKAMINE, S., TAKAHASHI, N., OSAKA, H., YAMAGATA, T., NAKAMURA, K., TSURUSAKI, Y., NAKASHIMA, M., MIYAKE, N., SHIINA, M., OGATA, K. & MATSUMOTO, N. 2016. Phenotypic spectrum of GNAO1 variants: epileptic encephalopathy to involuntary movements with severe developmental delay. *Eur J Hum Genet,* 24**,** 129-34.

SAITSU, H., KATO, M., KOIDE, A., GOTO, T., FUJITA, T., NISHIYAMA, K., TSURUSAKI, Y., DOI, H., MIYAKE, N., HAYASAKA, K. & MATSUMOTO, N. 2012. Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. *Ann Neurol,* 72**,** 298-300.

SAITSU, H., KATO, M., MIZUGUCHI, T., HAMADA, K., OSAKA, H., TOHYAMA, J., URUNO, K., KUMADA, S., NISHIYAMA, K., NISHIMURA, A., OKADA, I., YOSHIMURA, Y., HIRAI, S., KUMADA, T., HAYASAKA, K., FUKUDA, A., OGATA, K. & MATSUMOTO, N. 2008. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet,* 40**,** 782-8.

SAITSU, H., TOHYAMA, J., WALSH, T., KATO, M., KOBAYASHI, Y., LEE, M., TSURUSAKI, Y., MIYAKE, N., GOTO, Y., NISHINO, I., OHTAKE, A., KING, M. C. & MATSUMOTO, N. 2014a. A girl with West syndrome and autistic features harboring a de novo TBL1XR1 mutation. *J Hum Genet,* 59**,** 581-3.

SAITSU, H., YAMASHITA, S., TANAKA, Y., TSURUSAKI, Y., NAKASHIMA, M., MIYAKE, N. & MATSUMOTO, N. 2014b. Compound heterozygous BRAT1 mutations cause familial Ohtahara syndrome with hypertonia and microcephaly. *J Hum Genet,* 59**,** 687-90.

SAMOCHA, K. E., ROBINSON, E. B., SANDERS, S. J., STEVENS, C., SABO, A., MCGRATH, L. M., KOSMICKI, J. A., REHNSTRÖM, K., MALLICK, S., KIRBY, A., WALL, D. P., MACARTHUR, D. G., GABRIEL, S. B., DEPRISTO, M., PURCELL, S. M., PALOTIE, A., BOERWINKLE, E., BUXBAUM, J. D., COOK, E. H., JR., GIBBS, R. A., SCHELLENBERG,

G. D., SUTCLIFFE, J. S., DEVLIN, B., ROEDER, K., NEALE, B. M. & DALY, M. J. 2014. A framework for the interpretation of de novo mutation in human disease. *Nat Genet,* 46**,** 944-50.

SAMUELI, S., ABRAHAM, K., DRESSLER, A., GRÖPPEL, G., MÜHLEBNER-FAHRNGRUBER, A., SCHOLL, T., KASPRIAN, G., LACCONE, F. & FEUCHT, M. 2016. Efficacy and safety of Everolimus in children with TSC - associated epilepsy - Pilot data from an open single-center prospective study. *Orphanet J Rare Dis,* 11**,** 145.

SANDERS, S. J., CAMPBELL, A. J., COTTRELL, J. R., MOLLER, R. S., WAGNER, F. F., AULDRIDGE, A. L., BERNIER, R. A., CATTERALL, W. A., CHUNG, W. K., EMPFIELD, J. R., GEORGE, A. L., JR., HIPP, J. F., KHWAJA, O., KISKINIS, E., LAL, D., MALHOTRA, D., MILLICHAP, J. J., OTIS, T. S., PETROU, S., PITT, G., SCHUST, L. F., TAYLOR, C. M., TJERNAGEL, J., SPIRO, J. E. & BENDER, K. J. 2018. Progress in Understanding and Treating SCN2A-Mediated Disorders. *Trends Neurosci*.

SANDS, T. T., MICELI, F., LESCA, G., BECK, A. E., SADLEIR, L. G., ARRINGTON, D. K., SCHÖNEWOLF-GREULICH, B., MOUTTON, S., LAURITANO, A., NAPPI, P., SOLDOVIERI, M. V., SCHEFFER, I. E., MEFFORD, H. C., STONG, N., HEINZEN, E. L., GOLDSTEIN, D. B., PEREZ, A. G., KOSSOFF, E. H., STOCCO, A., SULLIVAN, J. A., SHASHI, V., GERARD, B., FRANCANNET, C., BISGAARD, A. M., TÜMER, Z., WILLEMS, M., RIVIER, F., VITOBELLO, A., THAKKAR, K., RAJAN, D. S., BARKOVICH, A. J., WECKHUYSEN, S., COOPER, E. C., TAGLIALATELA, M. & CILIO, M. R. 2019. Autism and developmental disability caused by KCNQ3 gain-of-function variants. *Ann Neurol,* 86**,** 181-192.

SANTOS, L. H., ANTONIUK, S. A., RODRIGUES, M., BRUNO, S. & BRUCK, I. 2002. Landau-Kleffner syndrome: study of four cases. *Arq Neuropsiquiatr,* 60**,** 239-41.

SAUDUBRAY, J. M. & GARCIA-CAZORLA, A. 2018. An overview of inborn errors of metabolism affecting the brain: from neurodevelopment to neurodegenerative disorders. *Dialogues Clin Neurosci,* 20**,** 301-325.

SAUNDERS, C. J., MILLER, N. A., SODEN, S. E., DINWIDDIE, D. L., NOLL, A., ALNADI, N. A., ANDRAWS, N., PATTERSON, M. L., KRIVOHLAVEK, L. A., FELLIS, J., HUMPHRAY, S., SAFFREY, P., KINGSBURY, Z., WEIR, J. C., BETLEY, J., GROCOCK, R. J., MARGULIES, E. H., FARROW, E. G., ARTMAN, M., SAFINA, N. P., PETRIKIN, J. E., HALL, K. P. & KINGSMORE, S. F. 2012. Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. *Sci Transl Med,* 4**,** 154ra135.

SCHANZE, D., KAYSERILI, H., SATKIN, B. N., ALTUNOGLU, U. & ZENKER, M. 2014. Fraser syndrome due to mutations in GRIP1--clinical phenotype in two families and expansion of the mutation spectrum. *Am J Med Genet A,* 164a**,** 837-40.

SCHEFFER, I. E., BERKOVIC, S., CAPOVILLA, G., CONNOLLY, M. B., FRENCH, J., GUILHOTO, L., HIRSCH, E., JAIN, S., MATHERN, G. W., MOSHE, S. L., NORDLI, D. R., PERUCCA, E., TOMSON, T., WIEBE, S., ZHANG, Y. H. & ZUBERI, S. M. 2017. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia,* 58**,** 512-521.

SCHEFFER, I. E., TURNER, S. J., DIBBENS, L. M., BAYLY, M. A., FRIEND, K., HODGSON, B., BURROWS, L., SHAW, M., WEI, C., ULLMANN, R., ROPERS, H. H., SZEPETOWSKI, P., HAAN, E., MAZARIB, A., AFAWI, Z., NEUFELD, M. Y., ANDREWS, P. I., WALLACE, G., KIVITY, S., LEV, D., LERMAN-SAGIE, T., DERRY, C. P., KORCZYN, A. D., GECZ, J., MULLEY, J. C. & BERKOVIC, S. F. 2008. Epilepsy and mental retardation limited to females: an under-recognized disorder. *Brain,* 131**,** 918-27.

SCHELTENS-DE BOER, M. 2009. Guidelines for EEG in encephalopathy related to ESES/CSWS in children. *Epilepsia,* 50 Suppl 7**,** 13-7.

SCHMIDTS, M., HOU, Y., CORTÉS, C. R., MANS, D. A., HUBER, C., BOLDT, K., PATEL, M., VAN REEUWIJK, J., PLAZA, J. M., VAN BEERSUM, S. E., YAP, Z. M., LETTEBOER, S. J., TAYLOR, S. P., HERRIDGE, W., JOHNSON, C. A., SCAMBLER, P. J., UEFFING, M., KAYSERILI, H., KRAKOW, D., KING, S. M., BEALES, P. L., AL-GAZALI, L., WICKING, C., CORMIER-DAIRE, V., ROEPMAN, R., MITCHISON, H. M. & WITMAN, G. B. 2015. TCTEX1D2 mutations underlie Jeune asphyxiating thoracic dystrophy with impaired retrograde intraflagellar transport. *Nat Commun,* 6**,** 7074.

SCHMITTGEN, T. D. & LIVAK, K. J. 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc,* 3**,** 1101-8.

SCHOCH, K., MENG, L., SZELINGER, S., BEARDEN, D. R., STRAY-PEDERSEN, A., BUSK, O. L., STONG, N., LISTON, E., COHN, R. D., SCAGLIA, F., ROSENFELD, J. A., TARPINIAN, J., SKRABAN, C. M., DEARDORFF, M. A., FRIEDMAN, J. N., AKDEMIR, Z. C., WALLEY, N., MIKATI, M. A., KRANZ, P. G., JASIEN, J., MCCONKIE-ROSELL, A., MCDONALD, M., WECHSLER, S. B., FREEMARK, M., KANSAGRA, S., FREEDMAN, S., BALI, D., MILLAN, F., BALE, S., NELSON, S. F., LEE, H., DORRANI, N., GOLDSTEIN, D. B., XIAO, R., YANG, Y., POSEY, J. E., MARTINEZ-AGOSTO, J. A., LUPSKI, J. R., WANGLER, M. F. & SHASHI, V. 2017. A Recurrent De Novo Variant in NACC1 Causes a Syndrome Characterized by Infantile Epilepsy, Cataracts, and Profound Developmental Delay. *Am J Hum Genet,* 100**,** 343-351.

SCHOONJANS, A. S., MEUWISSEN, M., REYNIERS, E., KOOY, F. & CEULEMANS, B. 2016. PLCB1 epileptic encephalopathies; Review and expansion of the phenotypic spectrum. *Eur J Paediatr Neurol,* 20**,** 474-9.

SCHORLING, D. C., DIETEL, T., EVERS, C., HINDERHOFER, K., KORINTHENBERG, R., EZZO, D., BONNEMANN, C. G. & KIRSCHNER, J. 2017. Expanding Phenotype of De Novo Mutations in GNAO1: Four New Cases and Review of Literature. *Neuropediatrics,* 48**,** 371-377.

SCHUBERT, J., SIEKIERSKA, A., LANGLOIS, M. & MAY, P. 2014. Mutations in STX1B, encoding a presynaptic protein, cause fever-associated epilepsy syndromes. 46**,** 1327-32.

SCHWARZ, J. M., COOPER, D. N., SCHUELKE, M. & SEELOW, D. 2014. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods,* 11**,** 361-2.

SCHWARZ, Y., OLEINIKOV, K., SCHINDELDECKER, B., WYATT, A., WEISSGERBE, P., FLOCKERZI, V., BOEHM, U., FREICHEL, M. & BRUNS, D. 2019. TRPC channels

regulate Ca2+-signaling and short-term plasticity of fast glutamatergic synapses. *PLoS Biol,* 17**,** e3000445.

SCOVILLE, W. B. & MILNER, B. 1957. Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry,* 20**,** 11-21.

SELTZER, L. E., MA, M., AHMED, S., BERTRAND, M., DOBYNS, W. B., WHELESS, J. & PACIORKOWSKI, A. R. 2014. Epilepsy and outcome in FOXG1-related disorders. *Epilepsia,* 55**,** 1292-300.

ŠERÝ, O., LOCHMAN, J., POVOVÁ, J., JANOUT, V., PLESNÍK, J. & BALCAR, V. J. 2015. Association between 5q23.2-located polymorphism of CTXN3 gene (Cortexin 3) and schizophrenia in European-Caucasian males; implications for the aetiology of schizophrenia. *Behav Brain Funct,* 11**,** 10.

SESSA, A., FAGNOCCHI, L., MASTROTOTARO, G., MASSIMINO, L., ZAGHI, M., INDRIGO, M., CATTANEO, S., MARTINI, D., GABELLINI, C., PUCCI, C., FASCIANI, A., BELLI, R., TAVERNA, S., ANDREAZZOLI, M., ZIPPO, A. & BROCCOLI, V. 2019. SETD5 Regulates Chromatin Methylation State and Preserves Global Transcriptional Fidelity during Brain Development and Neuronal Wiring. *Neuron,* 104**,** 271-289.e13.

SHA, L., WU, X., YAO, Y., WEN, B., FENG, J., SHA, Z., WANG, X., XING, X., DOU, W., JIN, L., LI, W., WANG, N., SHEN, Y., WANG, J., WU, L. & XU, Q. 2014. Notch signaling activation promotes seizure activity in temporal lobe epilepsy. *Mol Neurobiol,* 49**,** 633-44.

SHAFI, M. M., VERNET, M., KLOOSTER, D., CHU, C. J., BORIC, K., BARNARD, M. E., ROMATOSKI, K., WESTOVER, M. B., CHRISTODOULOU, J. A., GABRIELI, J. D., WHITFIELD-GABRIELI, S., PASCUAL-LEONE, A. & CHANG, B. S. 2015. Physiological consequences of abnormal connectivity in a developmental epilepsy. *Ann Neurol,* 77**,** 487-503.

SHAHEEN, R., AL TALA, S., EWIDA, N., ABOUELHODA, M. & ALKURAYA, F. S. 2016. Epileptic encephalopathy with continuous spike-and-wave during sleep maps to a homozygous truncating mutation in AMPA receptor component FRRS1L. *Clin Genet,* 90**,** 282-3.

SHAHEEN, R., SHAMSELDIN, H. E., LOUCKS, C. M., SEIDAHMED, M. Z., ANSARI, S., IBRAHIM KHALIL, M., AL-YACOUB, N., DAVIS, E. E., MOLA, N. A., SZYMANSKA, K., HERRIDGE, W., CHUDLEY, A. E., CHODIRKER, B. N., SCHWARTZENTRUBER, J., MAJEWSKI, J., KATSANIS, N., POIZAT, C., JOHNSON, C. A., PARBOOSINGH, J., BOYCOTT, K. M., INNES, A. M. & ALKURAYA, F. S. 2014. Mutations in CSPP1, encoding a core centrosomal protein, cause a range of ciliopathy phenotypes in humans. *Am J Hum Genet,* 94**,** 73-9.

SHAPIRO, M. B. & SENAPATHY, P. 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. *Nucleic Acids Res,* 15**,** 7155-74.

SHARMA, S. & PRASAD, A. N. 2017. Inborn Errors of Metabolism and Epilepsy: Current Understanding, Diagnosis, and Treatment Approaches. *Int J Mol Sci,* 18.

SHEN, J., GILMORE, E. C., MARSHALL, C. A., HADDADIN, M., REYNOLDS, J. J., EYAID, W., BODELL, A., BARRY, B., GLEASON, D., ALLEN, K., GANESH, V. S., CHANG, B. S., GRIX, A., HILL, R. S., TOPCU, M., CALDECOTT, K. W., BARKOVICH, A. J. & WALSH, C. A. 2010. Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. *Nat Genet,* 42**,** 245-9.

SHEN, K., MAO, Q., YIN, X., ZHANG, C., JIN, Y., DENG, A., GU, Z. & CHEN, B. 2018. NLRP3 Inflammasome Activation Leads to Epileptic Neuronal Apoptosis. *Curr Neurovasc Res,* 15**,** 276-281.

SHIMOJIMA, K., SUGAWARA, M., SHICHIJI, M., MUKAIDA, S., TAKAYAMA, R., IMAI, K. & YAMAMOTO, T. 2011. Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. *J Hum Genet,* 56**,** 561-5.

SHIMOYAMA, Y., TSUJIMOTO, G., KITAJIMA, M. & NATORI, M. 2000. Identification of three human type-II classic cadherins and frequent heterophilic interactions between different subclasses of type-II classic cadherins. *Biochem J,* 349**,** 159-67.

SHLEPER, M., KARTVELISHVILY, E. & WOLOSKER, H. 2005. D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J Neurosci,* 25**,** 9413-7.

SIA, G. M., CLEM, R. L. & HUGANIR, R. L. 2013. The human language-associated gene SRPX2 regulates synapse formation and vocalization in mice. *Science,* 342**,** 987-91.

SIBAROV, D. A., BRUNEAU, N., ANTONOV, S. M., SZEPETOWSKI, P., BURNASHEV, N. & GINIATULLIN, R. 2017. Functional Properties of Human NMDA Receptors Associated with Epilepsy-Related Mutations of GluN2A Subunit. *Front Cell Neurosci,* 11**,** 155.

SICCA, F., AMBROSINI, E., MARCHESE, M., SFORNA, L., SERVETTINI, I., VALVO, G., BRIGNONE, M. S., LANCIOTTI, A., MORO, F., GROTTESI, A., CATACUZZENO, L., BALDINI, S., HASAN, S., D'ADAMO, M. C., FRANCIOLINI, F., MOLINARI, P., SANTORELLI, F. M. & PESSIA, M. 2016. Gain-of-function defects of astrocytic Kir4.1 channels in children with autism spectrum disorders and epilepsy. *Sci Rep,* 6**,** 34325.

SIEKIERSKA, A., ISRIE, M., LIU, Y., SCHELDEMAN, C., VANTHILLO, N., LAGAE, L., DE WITTE, P. A., VAN ESCH, H., GOLDFARB, M. & BUYSE, G. M. 2016. Gain-of-function FHF1 mutation causes early-onset epileptic encephalopathy with cerebellar atrophy. *Neurology,* 86**,** 2162-70.

SIM, N. L., KUMAR, P., HU, J., HENIKOFF, S., SCHNEIDER, G. & NG, P. C. 2012. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res,* 40**,** W452-7.

SIMONS, C., GRIFFIN, L. B., HELMAN, G., GOLAS, G., PIZZINO, A., BLOOM, M., MURPHY, J. L., CRAWFORD, J., EVANS, S. H., TOPPER, S., WHITEHEAD, M. T., SCHREIBER, J. M., CHAPMAN, K. A., TIFFT, C., LU, K. B., GAMPER, H., SHIGEMATSU, M., TAFT, R. J., ANTONELLIS, A., HOU, Y. M. & VANDERVER, A. 2015. Loss-of-function alanyl-tRNA synthetase mutations cause an autosomal-recessive early-onset epileptic encephalopathy with persistent myelination defect. *Am J Hum Genet,* 96**,** 675-81.

SINCLAIR, D. B. & SNYDER, T. J. 2005. Corticosteroids for the treatment of Landau-kleffner syndrome and continuous spike-wave discharge during sleep. *Pediatr Neurol,* 32**,** 300-6.

SINGH, D., LAU, M., AYERS, T., SINGH, Y., AKINGBOLA, O., BARBIERO, L. & NELSON, S. 2016. De Novo Heterogeneous Mutations in SCN2A and GRIN2A Genes and Seizures With Ictal Vocalizations. *Clin Pediatr (Phila),* 55**,** 867-70.

SINGH, N. A., PAPPAS, C., DAHLE, E. J., CLAES, L. R., PRUESS, T. H., DE JONGHE, P., THOMPSON, J., DIXON, M., GURNETT, C., PEIFFER, A., WHITE, H. S., FILLOUX, F. & LEPPERT, M. F. 2009. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet,* 5**,** e1000649.

SINHA, V., UKKOLA-VUOTI, L., ORTEGA-ALONSO, A., TORNIAINEN-HOLM, M., THERMAN, S., TUULIO-HENRIKSSON, A., JYLHÄ, P., KAPRIO, J., HOVATTA, I., ISOMETSÄ, E., CANNON, T. D., LÖNNQVIST, J., PAUNIO, T., SUVISAARI, J. & HENNAH, W. 2019. Variants in regulatory elements of PDE4D associate with major mental illness in the Finnish population. *Mol Psychiatry*.

SLATKO, B. E., GARDNER, A. F. & AUSUBEL, F. M. 2018. Overview of Next-Generation Sequencing Technologies. *Curr Protoc Mol Biol,* 122**,** e59.

SMITH, L., SINGHAL, N., EL ACHKAR, C. M., TRUGLIO, G., ROSEN SHEIDLEY, B., SULLIVAN, J. & PODURI, A. 2018. PCDH19-related epilepsy is associated with a broad neurodevelopmental spectrum. *Epilepsia,* 59**,** 679-689.

SMOGAVEC, M., CLEALL, A., HOYER, J., LEDERER, D., NASSOGNE, M. C., PALMER, E. E., DEPREZ, M., BENOIT, V., MAYSTADT, I., NOAKES, C., LEAL, A., SHAW, M., GECZ, J., RAYMOND, L., REIS, A., SHEARS, D., BROCKMANN, K. & ZWEIER, C. 2016. Eight further individuals with intellectual disability and epilepsy carrying bi-allelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum. *J Med Genet,* 53**,** 820-827.

SNEHAVARDHAN, P., LAL, B. B., SOOD, V., KHANNA, R. & ALAM, S. 2019. Efficacy And Safety Of Sodium Benzoate In The Management Of Hyperammonemia in Decompensated Chronic Liver Disease of the Childhood- A Double Blind Randomised Controlled Trial. *J Pediatr Gastroenterol Nutr*.

SOKKA, A. L., MUDO, G., AALTONEN, J., BELLUARDO, N., LINDHOLM, D. & KORHONEN, L. 2005. Bruce/apollon promotes hippocampal neuron survival and is downregulated by kainic acid. *Biochem Biophys Res Commun,* 338**,** 729-35.

SOPRANO, A. M., GARCIA, E. F., CARABALLO, R. & FEJERMAN, N. 1994. Acquired epileptic aphasia: neuropsychologic follow-up of 12 patients. *Pediatr Neurol,* 11**,** 230-5.

SOTO, D., OLIVELLA, M., GRAU, C., ARMSTRONG, J., ALCON, C., GASULL, X., SANTOS-GOMEZ, A., LOCUBICHE, S., GOMEZ DE SALAZAR, M., GARCIA-DIAZ, R., GRATACOS-BATLLE, E., RAMOS-VICENTE, D., CHU-VAN, E., COLSCH, B., FERNANDEZ-DUENAS, V., CIRUELA, F., BAYES, A., SINDREU, C., LOPEZ-SALA, A., GARCIA-CAZORLA, A. & ALTAFAJ, X. 2019. l-Serine dietary supplementation is associated with clinical improvement of loss-of-function GRIN2B-related pediatric encephalopathy. *Sci Signal,* 12.

SOUTHGATE, L., SUKALO, M., KAROUNTZOS, A. S. V., TAYLOR, E. J., COLLINSON, C. S., RUDDY, D., SNAPE, K. M., DALLAPICCOLA, B., TOLMIE, J. L., JOSS, S., BRANCATI, F., DIGILIO, M. C., GRAUL-NEUMANN, L. M., SALVIATI, L., COERDT, W., JACQUEMIN, E., WUYTS, W., ZENKER, M., MACHADO, R. D. & TREMBATH, R. C. 2015. Haploinsufficiency of the NOTCH1 Receptor as a Cause of Adams-Oliver Syndrome With Variable Cardiac Anomalies. *Circ Cardiovasc Genet,* 8**,** 572-581.

STAM, A. H., LUIJCKX, G. J., POLL-THE, B. T., GINJAAR, I. B., FRANTS, R. R., HAAN, J., FERRARI, M. D., TERWINDT, G. M. & VAN DEN MAAGDENBERG, A. M. 2009. Early seizures and cerebral oedema after trivial head trauma associated with the CACNA1A S218L mutation. *J Neurol Neurosurg Psychiatry,* 80**,** 1125-9.

STEEL, D., SYMONDS, J. D., ZUBERI, S. M. & BRUNKLAUS, A. 2017. Dravet syndrome and its mimics: Beyond SCN1A. *Epilepsia,* 58**,** 1807-1816.

STEFANATOS, G. 2011. Changing perspectives on Landau-Kleffner syndrome. *Clin Neuropsychol,* 25**,** 963-88.

STENMARK, H., AASLAND, R. & DRISCOLL, P. C. 2002. The phosphatidylinositol 3-phosphate-binding FYVE finger. *FEBS Lett,* 513**,** 77-84.

STERIADE, C., FRENCH, J. & DEVINSKY, O. 2020. Epilepsy: key experimental therapeutics in early clinical development. *Expert Opin Investig Drugs,* 29**,** 373-383.

STIRNIMANN, C. U., PETSALAKI, E., RUSSELL, R. B. & MULLER, C. W. 2010. WD40 proteins propel cellular networks. *Trends Biochem Sci,* 35**,** 565-74.

STITTRICH, A. B., LEHMAN, A., BODIAN, D. L., ASHWORTH, J., ZONG, Z., LI, H., LAM, P., KHROMYKH, A., IYER, R. K., VOCKLEY, J. G., BAVEJA, R., SILVA, E. S., DIXON, J., LEON, E. L., SOLOMON, B. D., GLUSMAN, G., NIEDERHUBER, J. E., ROACH, J. C. & PATEL, M. S. 2014. Mutations in NOTCH1 cause Adams-Oliver syndrome. *Am J Hum Genet,* 95**,** 275-84.

STOCKINGER, J., STRZELCZYK, A., NEMECEK, A., CICANIC, M., BÖSEBECK, F., BRANDT, C., HAMER, H., INTRAVOOTH, T. & STEINHOFF, B. J. 2021. Everolimus in adult tuberous sclerosis complex patients with epilepsy: Too late for success? A retrospective study. *Epilepsia*.

STODBERG, T., MCTAGUE, A., RUIZ, A. J., HIRATA, H., ZHEN, J., LONG, P., FARABELLA, I., MEYER, E., KAWAHARA, A., VASSALLO, G., STIVAROS, S. M., BJURSELL, M. K.,

STRANNEHEIM, H., TIGERSCHIOLD, S., PERSSON, B., BANGASH, I., DAS, K., HUGHES, D., LESKO, N., LUNDEBERG, J., SCOTT, R. C., PODURI, A., SCHEFFER, I. E., SMITH, H. & GISSEN, P. 2015. Mutations in SLC12A5 in epilepsy of infancy with migrating focal seizures. 6**,** 8038.

STOGMANN, E., LICHTNER, P., BAUMGARTNER, C., BONELLI, S., ASSEM-HILGER, E., LEUTMEZER, F., SCHMIED, M., HOTZY, C., STROM, T. M., MEITINGER, T., ZIMPRICH, F. & ZIMPRICH, A. 2006. Idiopathic generalized epilepsy phenotypes associated with different EFHC1 mutations. *Neurology,* 67**,** 2029-31.

STRAUB, J., KONRAD, E. D. H., GRUNER, J., TOUTAIN, A., BOK, L. A., CHO, M. T., CRAWFORD, H. P., DUBBS, H., DOUGLAS, G., JOBLING, R., JOHNSON, D., KROCK, B., MIKATI, M. A., NESBITT, A., NICOLAI, J., PHILLIPS, M., PODURI, A., ORTIZ-GONZALEZ, X. R., POWIS, Z., SANTANI, A., SMITH, L., STEGMANN, A. P. A., STUMPEL, C., VREEBURG, M., FLIEDNER, A., GREGOR, A., STICHT, H. & ZWEIER, C. 2018. Missense Variants in RHOBTB2 Cause a Developmental and Epileptic Encephalopathy in Humans, and Altered Levels Cause Neurological Defects in Drosophila. *Am J Hum Genet,* 102**,** 44-57.

STRAUSSBERG, R., GANELIN-COHEN, E., GOLDBERG-STERN, H., TZUR, S., BEHAR, D. M., SMIRIN-YOSEF, P., SALMON-DIVON, M. & BASEL-VANAGAITE, L. 2015. Lethal neonatal rigidity and multifocal seizure syndrome--report of another family with a BRAT1 mutation. *Eur J Paediatr Neurol,* 19**,** 240-2.

STREHLOW, V., HEYNE, H. O., VLASKAMP, D. R. M., MARWICK, K. F. M., RUDOLF, G., DE BELLESCIZE, J., BISKUP, S., BRILSTRA, E. H., BROUWER, O. F., CALLENBACH, P. M. C., HENTSCHEL, J., HIRSCH, E., KIND, P. C., MIGNOT, C., PLATZER, K., RUMP, P., SKEHEL, P. A., WYLLIE, D. J. A., HARDINGHAM, G. E., VAN RAVENSWAAIJ-ARTS, C. M. A., LESCA, G. & LEMKE, J. R. 2019. GRIN2A-related disorders: genotype and functional consequence predict phenotype. *Brain,* 142**,** 80-92.

STREHLOW, V., HEYNE, H.O., LEMKE, J.R. 2015a. The Spectrum of GRIN2A-Associated Disorders. *Epileptologie,* 32**,** 147-151.

STREHLOW, V., HEYNE, HO, LEMKE, JR 2015b. The Spectrum of *GRIN2A*- Associated Disorders. *Epileptologie,* 32**,** 147-151.

SULEIMAN, J., ALLINGHAM-HAWKINS, D., HASHEM, M., SHAMSELDIN, H. E. & ALKURAYA, F. S. 2018. WDR45B-related intellectual disability, spastic quadriplegia, epilepsy, and cerebral hypoplasia: A consistent neurodevelopmental syndrome. 93**,** 360-364.

SULS, A., JAEHN, J. A., KECSKES, A., WEBER, Y., WECKHUYSEN, S., CRAIU, D. C., SIEKIERSKA, A., DJEMIE, T., AFRIKANOVA, T., GORMLEY, P., VON SPICZAK, S., KLUGER, G., ILIESCU, C. M., TALVIK, T., TALVIK, I., MERAL, C., CAGLAYAN, H. S., GIRALDEZ, B. G., SERRATOSA, J., LEMKE, J. R., HOFFMAN-ZACHARSKA, D., SZCZEPANIK, E., BARISIC, N., KOMAREK, V., HJALGRIM, H., MOLLER, R. S., LINNANKIVI, T., DIMOVA, P., STRIANO, P., ZARA, F., MARINI, C., GUERRINI, R., DEPIENNE, C., BAULAC, S., KUHLENBAUMER, G., CRAWFORD, A. D., LEHESJOKI, A. E., DE WITTE, P. A., PALOTIE, A., LERCHE, H., ESGUERRA, C. V., DE JONGHE, P.

& HELBIG, I. 2013. De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. *Am J Hum Genet,* 93**,** 967-75.

SUREL, C., GUILLET, M., LENOIR, M., BOURIEN, J., SENDIN, G., JOLY, W., DELPRAT, B., LESPERANCE, M. M., PUEL, J. L. & NOUVIAN, R. 2016. Remodeling of the Inner Hair Cell Microtubule Meshwork in a Mouse Model of Auditory Neuropathy AUNA1. *eNeuro,* 3.

SURGUCHEV, A. A. & SURGUCHOV, A. 2020. ABCA7-A Member of the ABC Transporter Family in Healthy and Ailing Brain. *Brain Sci,* 10.

SUZUKI-MUROMOTO, S., WAKUSAWA, K., MIYABAYASHI, T., SATO, R., OKUBO, Y., ENDO, W., INUI, T., TOGASHI, N., KATO, A., OBA, H., NAKASHIMA, M. & SAITSU, H. 2018. A case of new PCDH12 gene variants presented as dyskinetic cerebral palsy with epilepsy. 63**,** 749-753.

SWANGER, S. A., CHEN, W., WELLS, G., BURGER, P. B., TANKOVIC, A., BHATTACHARYA, S., STRONG, K. L., HU, C., KUSUMOTO, H., ZHANG, J., ADAMS, D. R., MILLICHAP, J. J., PETROVSKI, S., TRAYNELIS, S. F. & YUAN, H. 2016. Mechanistic Insight into NMDA Receptor Dysregulation by Rare Variants in the GluN2A and GluN2B Agonist Binding Domains. *Am J Hum Genet,* 99**,** 1261-1280.

SWANN, J. W., SMITH, K. L. & BRADY, R. J. 1990. Neural networks and synaptic transmission in immature hippocampus. *Adv Exp Med Biol,* 268**,** 161-71.

SWEATT, J. D. 2016. Neural plasticity and behavior - sixty years of conceptual advances. *J Neurochem,* 139 Suppl 2**,** 179-199.

SYMONDS, J. D., JOSS, S., METCALFE, K. A., SOMARATHI, S., CRUDEN, J., DEVLIN, A. M., DONALDSON, A., DIDONATO, N., FITZPATRICK, D., KAISER, F. J., LAMPE, A. K., LEES, M. M., MCLELLAN, A., MONTGOMERY, T., MUNDADA, V., NAIRN, L., SARKAR, A., SCHALLNER, J., POZOJEVIC, J., PARENTI, I., TAN, J., TURNPENNY, P., WHITEHOUSE, W. P. & ZUBERI, S. M. 2017. Heterozygous truncation mutations of the SMC1A gene cause a severe early onset epilepsy with cluster seizures in females: Detailed phenotyping of 10 new cases. *Epilepsia,* 58**,** 565-575.

SYRBE, S., HARMS, F. L., PARRINI, E., MONTOMOLI, M., MUTZE, U., HELBIG, K. L., POLSTER, T., ALBRECHT, B., BERNBECK, U., VAN BINSBERGEN, E., BISKUP, S., BURGLEN, L., DENECKE, J., HERON, B., HEYNE, H. O., HOFFMANN, G. F., HORNEMANN, F., MATSUSHIGE, T., MATSUURA, R., KATO, M., KORENKE, G. C., KUECHLER, A., LAMMER, C., MERKENSCHLAGER, A., MIGNOT, C., RUF, S., NAKASHIMA, M., SAITSU, H., STAMBERGER, H., PISANO, T., TOHYAMA, J., WECKHUYSEN, S., WERCKX, W., WICKERT, J., MARI, F., VERBEEK, N. E., MOLLER, R. S., KOELEMAN, B., MATSUMOTO, N., DOBYNS, W. B., BATTAGLIA, D., LEMKE, J. R., KUTSCHE, K. & GUERRINI, R. 2017. Delineating SPTAN1 associated phenotypes: from isolated epilepsy to encephalopathy with progressive brain atrophy. *Brain,* 140**,** 2322-2336.

SYRBE, S., HEDRICH, U. B. S., RIESCH, E., DJEMIE, T., MULLER, S., MOLLER, R. S., MAHER, B., HERNANDEZ-HERNANDEZ, L., SYNOFZIK, M., CAGLAYAN, H. S., ARSLAN, M., SERRATOSA, J. M., NOTHNAGEL, M., MAY, P., KRAUSE, R., LOFFLER, H., DETERT, K., DORN, T., VOGT, H., KRAMER, G., SCHOLS, L., MULLIS, P. E., LINNANKIVI, T., LEHESJOKI, A. E., STERBOVA, K., CRAIU, D. C., HOFFMAN-ZACHARSKA, D., KORFF, C. M., WEBER, Y. G., STEINLIN, M., GALLATI, S., BERTSCHE, A., BERNHARD, M. K., MERKENSCHLAGER, A., KIESS, W., GONZALEZ, M., ZUCHNER, S., PALOTIE, A., SULS, A., DE JONGHE, P., HELBIG, I., BISKUP, S., WOLFF, M., MALJEVIC, S., SCHULE, R., SISODIYA, S. M., WECKHUYSEN, S., LERCHE, H. & LEMKE, J. R. 2015. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. *Nat Genet,* 47**,** 393-399.

TAKEGUCHI, R., HAGINOYA, K., UCHIYAMA, Y., FUJITA, A., NAGURA, M., TAKESHITA, E., INUI, T., OKUBO, Y., SATO, R., MIYABAYASHI, T., TOGASHI, N., SAITO, T., NAKAGAWA, E., SUGAI, K., NAKASHIMA, M., SAITSU, H., MATSUMOTO, N. & SASAKI, M. 2018. Two Japanese cases of epileptic encephalopathy associated with an FGF12 mutation. *Brain Dev*.

TALEBIAN, A. & HENKEMEYER, M. 2019. EphB2 receptor cell-autonomous forward signaling mediates auditory memory recall and learning-driven spinogenesis. *Commun Biol,* 2**,** 372.

TALKOWSKI, M. E., MULLEGAMA, S. V., ROSENFELD, J. A., VAN BON, B. W., SHEN, Y., REPNIKOVA, E. A., GASTIER-FOSTER, J., THRUSH, D. L., KATHIRESAN, S., RUDERFER, D. M., CHIANG, C., HANSCOM, C., ERNST, C., LINDGREN, A. M., MORTON, C. C., AN, Y., ASTBURY, C., BRUETON, L. A., LICHTENBELT, K. D., ADES, L. C., FICHERA, M., ROMANO, C., INNIS, J. W., WILLIAMS, C. A., BARTHOLOMEW, D., VAN ALLEN, M. I., PARIKH, A., ZHANG, L., WU, B. L., PYATT, R. E., SCHWARTZ, S., SHAFFER, L. G., DE VRIES, B. B., GUSELLA, J. F. & ELSEA, S. H. 2011. Assessment of 2q23.1 microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder. *Am J Hum Genet,* 89**,** 551-63.

TANG, R., PROSSER, D. O. & LOVE, D. R. 2016. Evaluation of Bioinformatic Programmes for the Analysis of Variants within Splice Site Consensus Regions. *Adv Bioinformatics,* 2016**,** 5614058.

TANG, S. J., REIS, G., KANG, H., GINGRAS, A. C., SONENBERG, N. & SCHUMAN, E. M. 2002. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc Natl Acad Sci U S A,* 99**,** 467-72.

TAO, H., MANAK, J. R., SOWERS, L., MEI, X., KIYONARI, H., ABE, T., DAHDALEH, N. S., YANG, T., WU, S., CHEN, S., FOX, M. H., GURNETT, C., MONTINE, T., BIRD, T., SHAFFER, L. G., ROSENFELD, J. A., MCCONNELL, J., MADAN-KHETARPAL, S., BERRY-KRAVIS, E., GRIESBACH, H., SANETO, R. P., SCOTT, M. P., ANTIC, D., REED, J., BOLAND, R., EHAIDEB, S. N., EL-SHANTI, H., MAHAJAN, V. B., FERGUSON, P. J., AXELROD, J. D., LEHESJOKI, A. E., FRITZSCH, B., SLUSARSKI, D. C., WEMMIE, J., UENO, N. & BASSUK, A. G. 2011. Mutations in prickle orthologs cause seizures in flies, mice, and humans. *Am J Hum Genet,* 88**,** 138-49.

TAO, J., VAN ESCH, H., HAGEDORN-GREIWE, M., HOFFMANN, K., MOSER, B., RAYNAUD, M., SPERNER, J., FRYNS, J. P., SCHWINGER, E., GECZ, J., ROPERS, H. H. & KALSCHEUER, V. M. 2004. Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation. *Am J Hum Genet,* 75**,** 1149-54.

TAPIE, A., PI-DENIS, N., SOUTO, J., VOMERO, A., PELUFFO, G., BOIDI, M., CIGANDA, M., CURBELO, N., RAGGIO, V., ROCHE, L. & PASTRO, L. 2017. A novel mutation in the OAR domain of the ARX gene. *Clin Case Rep,* 5**,** 170-174.

TARABEUX, J., KEBIR, O., GAUTHIER, J., HAMDAN, F. F., XIONG, L., PITON, A., SPIEGELMAN, D., HENRION, E., MILLET, B., FATHALLI, F., JOOBER, R., RAPOPORT, J. L., DELISI, L. E., FOMBONNE, E., MOTTRON, L., FORGET-DUBOIS, N., BOIVIN, M., MICHAUD, J. L., DRAPEAU, P., LAFRENIERE, R. G., ROULEAU, G. A. & KREBS, M. O. 2011. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. *Transl Psychiatry,* 1**,** e55.

TARTA-ARSENE, O., BARCA, D., CRAIU, D. & ILIESCU, C. 2017. Practical clues for diagnosing WWOX encephalopathy. *Epileptic Disord,* 19**,** 357-361.

TASSINARI, C. A. & RUBBOLI, G. 2019. Encephalopathy related to Status Epilepticus during slow Sleep: current concepts and future directions. *Epileptic Disord,* 21**,** 82-87.

TEMPLIN, C., GHADRI, J. R., ROUGIER, J. S., BAUMER, A., KAPLAN, V., ALBESA, M., STICHT, H., RAUCH, A., PULEO, C., HU, D., BARAJAS-MARTINEZ, H., ANTZELEVITCH, C., LÜSCHER, T. F., ABRIEL, H. & DURU, F. 2011. Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). *Eur Heart J,* 32**,** 1077-88.

THE DECIPHERING DEVELOPMENTAL DISORDERS STUDY 2015. Large-scale discovery of novel genetic causes of developmental disorders. *Nature,* 519**,** 223-8.

THE DECIPHERING DEVELOPMENTAL DISORDERS STUDY 2017. Prevalence and architecture of de novo mutations in developmental disorders. *Nature,* 542**,** 433-438.

THEVENON, J., MILH, M., FEILLET, F., ST-ONGE, J., DUFFOURD, Y., JUGE, C., ROUBERTIE, A., HERON, D., MIGNOT, C., RAFFO, E., ISIDOR, B., WAHLEN, S., SANLAVILLE, D., VILLENEUVE, N., DARMENCY-STAMBOUL, V., TOUTAIN, A., LEFEBVRE, M., CHOUCHANE, M., HUET, F., LAFON, A., DE SAINT MARTIN, A., LESCA, G., EL CHEHADEH, S., THAUVIN-ROBINET, C., MASUREL-PAULET, A., ODENT, S., VILLARD, L., PHILIPPE, C., FAIVRE, L. & RIVIERE, J. B. 2014. Mutations in SLC13A5 cause autosomal-recessive epileptic encephalopathy with seizure onset in the first days of life. *Am J Hum Genet,* 95**,** 113-20.

TOHYAMA, J., AKASAKA, N., OSAKA, H., MAEGAKI, Y., KATO, M., SAITO, N., YAMASHITA, S. & OHNO, K. 2008. Early onset West syndrome with cerebral hypomyelination and reduced cerebral white matter. *Brain Dev,* 30**,** 349-55.

TOM DIECK, S., SANMARTI-VILA, L., LANGNAESE, K., RICHTER, K., KINDLER, S., SOYKE, A., WEX, H., SMALLA, K. H., KAMPF, U., FRANZER, J. T., STUMM, M., GARNER, C. C. & GUNDELFINGER, E. D. 1998. Bassoon, a novel zinc-finger CAG/glutamine-repeat protein selectively localized at the active zone of presynaptic nerve terminals. *J Cell Biol,* 142**,** 499-509.

TORKAMANI, A., BERSELL, K., JORGE, B. S., BJORK, R. L., JR., FRIEDMAN, J. R., BLOSS, C. S., COHEN, J., GUPTA, S., NAIDU, S., VANOYE, C. G., GEORGE, A. L., JR. & KEARNEY, J. A. 2014. De novo KCNB1 mutations in epileptic encephalopathy. *Ann Neurol,* 76**,** 529-540.

TOSO, V., MOSCHINI, M., GAGNIN, G. & ANTONI, D. 1981. [Acquired aphasia of the epileptic child. 3 cases and review of the literature]. *Rev Neurol (Paris),* 137**,** 425-34.

TRABZUNI, D., RYTEN, M., WALKER, R., SMITH, C., IMRAN, S., RAMASAMY, A., WEALE, M. E. & HARDY, J. 2011. Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. *J Neurochem,* 119**,** 275-82.

TRAYNELIS, S. F., WOLLMUTH, L. P., MCBAIN, C. J., MENNITI, F. S., VANCE, K. M., OGDEN, K. K., HANSEN, K. B., YUAN, H., MYERS, S. J. & DINGLEDINE, R. 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev,* 62**,** 405-96.

TREMINO, L. & FORCADA-NADAL, A. 2018. Insight into vitamin B6 -dependent epilepsy due to PLPBP (previously PROSC) missense mutations. 39**,** 1002-1013.

TRIPATHI, M., DIXIT, A. & CHANDRA, P. S. 2016. Galectin-3, an important yet unexplored molecule in drug resistant epilepsy. *Neurol India,* 64**,** 237-8.

TROTMAN, M., BARAD, Z., GUEVREMONT, D., WILLIAMS, J. & LEITCH, B. 2014. Changes in the GRIP 1&2 scaffolding proteins in the cerebellum of the ataxic stargazer mouse. *Brain Res,* 1546**,** 53-62.

TRUMP, N., MCTAGUE, A., BRITTAIN, H., PAPANDREOU, A., MEYER, E., NGOH, A., PALMER, R., MORROGH, D., BOUSTRED, C., HURST, J. A., JENKINS, L., KURIAN, M. A. & SCOTT, R. H. 2016. Improving diagnosis and broadening the phenotypes in early-onset seizure and severe developmental delay disorders through gene panel analysis. *J Med Genet,* 53**,** 310-7.

TSAI, G. E. 2016. Ultimate Translation: Developing Therapeutics Targeting on N-Methyl-d-Aspartate Receptor. *Adv Pharmacol,* 76**,** 257-309.

TSAI, G. E., FALK, W. E., GUNTHER, J. & COYLE, J. T. 1999. Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. *Am J Psychiatry,* 156**,** 467-9.

TSAI, M. H., CHAN, C. K., CHANG, Y. C., LIN, C. H., LIOU, C. W., CHANG, W. N., NG, C. C., LIM, K. S. & HWANG, D. Y. 2018. Molecular Genetic Characterization of Patients

With Focal Epilepsy Using a Customized Targeted Resequencing Gene Panel. *Front Neurol,* 9**,** 515.

TSURU, T., MORI, M., MIZUGUCHI, M. & MOMOI, M. Y. 2000. Effects of high-dose intravenous corticosteroid therapy in Landau-Kleffner syndrome. *Pediatr Neurol,* 22**,** 145-7.

TUFT, M., ARVA, M., BJORNVOLD, M., WILSON, J. A. & NAKKEN, K. O. 2015. Landau-Kleffner syndrome. *Tidsskr Nor Laegeforen,* 135**,** 2061-4.

TURNER, S. J., MAYES, A. K., VERHOEVEN, A., MANDELSTAM, S. A., MORGAN, A. T. & SCHEFFER, I. E. 2015. GRIN2A: an aptly named gene for speech dysfunction. *Neurology,* 84**,** 586-93.

URFER, R., MOEBIUS, H. J., SKOLOUDIK, D., SANTAMARINA, E., SATO, W., MITA, S. & MUIR, K. W. 2014. Phase II trial of the Sigma-1 receptor agonist cutamesine (SA4503) for recovery enhancement after acute ischemic stroke. *Stroke,* 45**,** 3304-10.

VALLIANATOS, C. N. & IWASE, S. 2015. Disrupted intricacy of histone H3K4 methylation in neurodevelopmental disorders. *Epigenomics,* 7**,** 503-19.

VAN BOGAERT, P. 2013. Epileptic encephalopathy with continuous spike-waves during slow-wave sleep including Landau-Kleffner syndrome. *Handb Clin Neurol,* 111**,** 635-40.

VAN DEN MUNCKHOF, B., ALDERWEIRELD, C., DAVELAAR, S., VAN TEESELING, H. C., NIKOLAKOPOULOS, S., BRAUN, K. P. J. & JANSEN, F. E. 2018. Treatment of electrical status epilepticus in sleep: Clinical and EEG characteristics and response to 147 treatments in 47 patients. *Eur J Paediatr Neurol,* 22**,** 64-71.

VAN DEN MUNCKHOF, B., ARZIMANOGLOU, A., PERUCCA, E., VAN TEESELING, H. C., LEIJTEN, F. S. S., BRAUN, K. P. J. & JANSEN, F. E. 2020. Corticosteroids versus clobazam in epileptic encephalopathy with ESES: a European multicentre randomised controlled clinical trial (RESCUE ESES*). *Trials,* 21**,** 957.

VAN DEN MUNCKHOF, B., VAN DEE, V., SAGI, L., CARABALLO, R. H., VEGGIOTTI, P., LIUKKONEN, E., LODDENKEMPER, T., SÁNCHEZ FERNÁNDEZ, I., BUZATU, M., BULTEAU, C., BRAUN, K. P. & JANSEN, F. E. 2015. Treatment of electrical status epilepticus in sleep: A pooled analysis of 575 cases. *Epilepsia,* 56**,** 1738-46.

VAN HENGEL, J., CALORE, M., BAUCE, B., DAZZO, E., MAZZOTTI, E., DE BORTOLI, M., LORENZON, A., LI MURA, I. E., BEFFAGNA, G., RIGATO, I., VLEESCHOUWERS, M., TYBERGHEIN, K., HULPIAU, P., VAN HAMME, E., ZAGLIA, T., CORRADO, D., BASSO, C., THIENE, G., DALIENTO, L., NAVA, A., VAN ROY, F. & RAMPAZZO, A. 2013. Mutations in the area composita protein αT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J,* 34**,** 201-10.

VARGHA-KHADEM, F., GADIAN, D. G., COPP, A. & MISHKIN, M. 2005. FOXP2 and the neuroanatomy of speech and language. *Nat Rev Neurosci,* 6**,** 131-8.

VASHISHTA, A., SLOMNICKI, L. P., PIETRZAK, M., SMITH, S. C., KOLIKONDA, M., NAIK, S. P., PARLATO, R. & HETMAN, M. 2018. RNA Polymerase 1 Is Transiently Regulated by Seizures and Plays a Role in a Pharmacological Kindling Model of Epilepsy. *Mol Neurobiol,* 55**,** 8374-8387.

VEERAMAH, K. R., JOHNSTONE, L., KARAFET, T. M., WOLF, D., SPRISSLER, R., SALOGIANNIS, J., BARTH-MARON, A., GREENBERG, M. E., STUHLMANN, T., WEINERT, S., JENTSCH, T. J., PAZZI, M., RESTIFO, L. L., TALWAR, D., ERICKSON, R. P. & HAMMER, M. F. 2013. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. *Epilepsia,* 54**,** 1270-81.

VEERAMAH, K. R., O'BRIEN, J. E., MEISLER, M. H., CHENG, X., DIB-HAJJ, S. D., WAXMAN, S. G., TALWAR, D., GIRIRAJAN, S., EICHLER, E. E., RESTIFO, L. L., ERICKSON, R. P. & HAMMER, M. F. 2012. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. *Am J Hum Genet,* 90**,** 502-10.

VEGGIOTTI, P., PERA, M. C., TEUTONICO, F., BRAZZO, D., BALOTTIN, U. & TASSINARI, C. A. 2012. Therapy of encephalopathy with status epilepticus during sleep (ESES/CSWS syndrome): an update. *Epileptic Disord,* 14**,** 1-11.

VERGULT, S., DHEEDENE, A., MEURS, A., FAES, F., ISIDOR, B., JANSSENS, S., GAUTIER, A., LE CAIGNEC, C. & MENTEN, B. 2015. Genomic aberrations of the CACNA2D1 gene in three patients with epilepsy and intellectual disability. *Eur J Hum Genet,* 23**,** 628-32.

VILLENEUVE, N., ABIDI, A., CACCIAGLI, P., MIGNON-RAVIX, C., CHABROL, B., VILLARD, L. & MILH, M. 2017. Heterogeneity of FHF1 related phenotype: Novel case with early onset severe attacks of apnea, partial mitochondrial respiratory chain complex II deficiency, neonatal onset seizures without neurodegeneration. *Eur J Paediatr Neurol,* 21**,** 783-786.

VIOLA, H., BALLARINI, F., MARTINEZ, M. C. & MONCADA, D. 2014. The tagging and capture hypothesis from synapse to memory. *Prog Mol Biol Transl Sci,* 122**,** 391-423.

VLASKAMP, D. R., RUMP, P., CALLENBACH, P. M., VOS, Y. J., SIKKEMA-RADDATZ, B., VAN RAVENSWAAIJ-ARTS, C. M. & BROUWER, O. F. 2016. Haploinsufficiency of the STX1B gene is associated with myoclonic astatic epilepsy. *Eur J Paediatr Neurol,* 20**,** 489-92.

VON SPICZAK, S., HELBIG, K. L., SHINDE, D. N., HUETHER, R., PENDZIWIAT, M., LOURENCO, C., NUNES, M. E., SARCO, D. P., KAPLAN, R. A., DLUGOS, D. J., KIRSCH, H., SLAVOTINEK, A., CILIO, M. R., CERVENKA, M. C., COHEN, J. S., MCCLELLAN, R., FATEMI, A., YUEN, A., SAGAWA, Y., LITTLEJOHN, R., MCLEAN, S. D., HERNANDEZ-HERNANDEZ, L., MAHER, B., MOLLER, R. S., PALMER, E., LAWSON, J. A., CAMPBELL, C. A., JOSHI, C. N., KOLBE, D. L., HOLLINGSWORTH, G., NEUBAUER, B. A., MUHLE, H., STEPHANI, U., SCHEFFER, I. E., PENA, S. D. J., SISODIYA, S. M. & HELBIG, I. 2017. DNM1 encephalopathy: A new disease of vesicle fission. *Neurology,* 89**,** 385-394.

VON STULPNAGEL, C., ENSSLEN, M., MOLLER, R. S., PAL, D. K., MASNADA, S., VEGGIOTTI, P., PIAZZA, E., DREESMANN, M., HARTLIEB, T., HERBERHOLD, T., HUGHES, E., KOCH, M., KUTZER, C., HOERTNAGEL, K., NITANDA, J., POHL, M., ROSTASY, K., HAACK, T. B., STOHR, K., KLUGER, G. & BORGGRAEFE, I. 2017. Epilepsy in patients with GRIN2A alterations: Genetics, neurodevelopment, epileptic phenotype and response to anticonvulsive drugs. *Eur J Paediatr Neurol,* 21**,** 530-541.

VON STULPNAGEL, C., FUNKE, C., HABERL, C., HORTNAGEL, K., JUNGLING, J., WEBER, Y. G., STAUDT, M. & KLUGER, G. 2015. SYNGAP1 Mutation in Focal and Generalized Epilepsy: A Literature Overview and A Case Report with Special Aspects of the EEG. *Neuropediatrics,* 46**,** 287-91.

VORHEES, C. V. & WILLIAMS, M. T. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc,* 1**,** 848-58.

VORSTMAN, J. A., VAN DAALEN, E., JALALI, G. R., SCHMIDT, E. R., PASTERKAMP, R. J., DE JONGE, M., HENNEKAM, E. A., JANSON, E., STAAL, W. G., VAN DER ZWAAG, B., BURBACH, J. P., KAHN, R. S., EMANUEL, B. S., VAN ENGELAND, H. & OPHOFF, R. A. 2011. A double hit implicates DIAPH3 as an autism risk gene. *Mol Psychiatry,* 16**,** 442-51.

VRECAR, I., INNES, J., JONES, E. A., KINGSTON, H., REARDON, W., KERR, B., CLAYTON-SMITH, J. & DOUZGOU, S. 2017. Further Clinical Delineation of the MEF2C Haploinsufficiency Syndrome: Report on New Cases and Literature Review of Severe Neurodevelopmental Disorders Presenting with Seizures, Absent Speech, and Involuntary Movements. *J Pediatr Genet,* 6**,** 129-141.

VUILLAUME, M. L., JEANNE, M., XUE, L., BLESSON, S. & DENOMME-PICHON, A. S. 2018. A novel mutation in the transmembrane 6 domain of GABBR2 leads to a Rett-like phenotype. 83**,** 437-439.

VUONG, C. K., WEI, W., LEE, J. A., LIN, C. H., DAMIANOV, A., DE LA TORRE-UBIETA, L., HALABI, R., OTIS, K. O., MARTIN, K. C., O'DELL, T. J. & BLACK, D. L. 2018. Rbfox1 Regulates Synaptic Transmission through the Inhibitory Neuron-Specific vSNARE Vamp1. *Neuron,* 98**,** 127-141.e7.

WAKAI, S., ITO, N., UEDA, D. & CHIBA, S. 1997. Landau-Kleffner syndrome and sulthiame. *Neuropediatrics,* 28**,** 135-6.

WALLACE, R. H., HODGSON, B. L., GRINTON, B. E., GARDINER, R. M., ROBINSON, R., RODRIGUEZ-CASERO, V., SADLEIR, L., MORGAN, J., HARKIN, L. A., DIBBENS, L. M., YAMAMOTO, T., ANDERMANN, E., MULLEY, J. C., BERKOVIC, S. F. & SCHEFFER, I. E. 2003. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology,* 61**,** 765-9.

WAMSLEY, B., JAGLIN, X. H., FAVUZZI, E., QUATTROCOLO, G., NIGRO, M. J., YUSUF, N., KHODADADI-JAMAYRAN, A., RUDY, B. & FISHELL, G. 2018. Rbfox1 Mediates Cell-type-Specific Splicing in Cortical Interneurons. *Neuron,* 100**,** 846-859.e7.

WANG, H., GAUR, U., XIAO, J., XU, B., XU, J. & ZHENG, W. 2018. Targeting phosphodiesterase 4 as a potential therapeutic strategy for enhancing neuroplasticity following ischemic stroke. *Int J Biol Sci,* 14**,** 1745-1754.

WANG, M. Y., LIU, X. Z., WANG, J. & WU, L. W. 2014. A novel mutation of the nicotinic acetylcholine receptor gene CHRNA4 in a Chinese patient with non-familial nocturnal frontal lobe epilepsy. *Epilepsy Res,* 108**,** 1927-31.

WANG, T., GUO, H., XIONG, B., STESSMAN, H. A., WU, H., COE, B. P., TURNER, T. N., LIU, Y., ZHAO, W., HOEKZEMA, K., VIVES, L., XIA, L., TANG, M., OU, J., CHEN, B., SHEN, Y., XUN, G., LONG, M., LIN, J., KRONENBERG, Z. N., PENG, Y., BAI, T., LI, H., KE, X., HU, Z., ZHAO, J., ZOU, X., XIA, K. & EICHLER, E. E. 2016. De novo genic mutations among a Chinese autism spectrum disorder cohort. *Nat Commun,* 7**,** 13316.

WANG, Z., EDWARDS, J. G., RILEY, N., PROVANCE, D. W., JR., KARCHER, R., LI, X. D., DAVISON, I. G., IKEBE, M., MERCER, J. A., KAUER, J. A. & EHLERS, M. D. 2008. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell,* 135**,** 535-48.

WEAVING, L. S., CHRISTODOULOU, J., WILLIAMSON, S. L., FRIEND, K. L., MCKENZIE, O. L., ARCHER, H., EVANS, J., CLARKE, A., PELKA, G. J., TAM, P. P., WATSON, C., LAHOOTI, H., ELLAWAY, C. J., BENNETTS, B., LEONARD, H. & GECZ, J. 2004. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet,* 75**,** 1079-93.

WEBER, J., FRINGS, L., RIJNTJES, M., URBACH, H., FISCHER, J., WEILLER, C., MEYER, P. T. & KLEBE, S. 2018. Chorea-Acanthocytosis Presenting as Autosomal Recessive Epilepsy in a Family With a Novel VPS13A Mutation. *Front Neurol,* 9**,** 1168.

WECKHUYSEN, S., MANDELSTAM, S., SULS, A., AUDENAERT, D., DECONINCK, T., CLAES, L. R., DEPREZ, L., SMETS, K., HRISTOVA, D., YORDANOVA, I., JORDANOVA, A., CEULEMANS, B., JANSEN, A., HASAERTS, D., ROELENS, F., LAGAE, L., YENDLE, S., STANLEY, T., HERON, S. E., MULLEY, J. C., BERKOVIC, S. F., SCHEFFER, I. E. & DE JONGHE, P. 2012. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann Neurol,* 71**,** 15-25.

WEI, F., YAN, L. M., SU, T., HE, N., LIN, Z. J., WANG, J., SHI, Y. W., YI, Y. H. & LIAO, W. P. 2017. Ion Channel Genes and Epilepsy: Functional Alteration, Pathogenic Potential, and Mechanism of Epilepsy. *Neurosci Bull,* 33**,** 455-477.

WEISS, L. A., ESCAYG, A., KEARNEY, J. A., TRUDEAU, M., MACDONALD, B. T., MORI, M., REICHERT, J., BUXBAUM, J. D. & MEISLER, M. H. 2003. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry,* 8**,** 186-94.

WIBOM, R., LASORSA, F. M., TOHONEN, V., BARBARO, M., STERKY, F. H., KUCINSKI, T., NAESS, K., JONSSON, M., PIERRI, C. L., PALMIERI, F. & WEDELL, A. 2009. AGC1 deficiency associated with global cerebral hypomyelination. *N Engl J Med,* 361**,** 489-95.

WILLIAMS, M. E., WILKE, S. A., DAGGETT, A., DAVIS, E., OTTO, S., RAVI, D., RIPLEY, B., BUSHONG, E. A., ELLISMAN, M. H., KLEIN, G. & GHOSH, A. 2011. Cadherin-9

regulates synapse-specific differentiation in the developing hippocampus. *Neuron,* 71**,** 640-55.

WINNEPENNINCKX, B., DEBACKER, K., RAMSAY, J., SMEETS, D., SMITS, A., FITZPATRICK, D. R. & KOOY, R. F. 2007. CGG-repeat expansion in the DIP2B gene is associated with the fragile site FRA12A on chromosome 12q13.1. *Am J Hum Genet,* 80**,** 221-31.

WIRRELL, E., HO, A. W. & HAMIWKA, L. 2006. Sulthiame therapy for continuous spike and wave in slow-wave sleep. *Pediatr Neurol,* 35**,** 204-8.

WOLFF, M., JOHANNESEN, K. M., HEDRICH, U. B. S., MASNADA, S., RUBBOLI, G., GARDELLA, E., LESCA, G., VILLE, D., MILH, M., VILLARD, L., AFENJAR, A., CHANTOT-BASTARAUD, S., MIGNOT, C., LARDENNOIS, C., NAVA, C., SCHWARZ, N., GERARD, M., PERRIN, L., DOUMMAR, D., AUVIN, S., MIRANDA, M. J., HEMPEL, M., BRILSTRA, E., KNOERS, N., VERBEEK, N., VAN KEMPEN, M., BRAUN, K. P., MANCINI, G., BISKUP, S., HORTNAGEL, K., DOCKER, M., BAST, T., LODDENKEMPER, T., WONG-KISIEL, L., BAUMEISTER, F. M., FAZELI, W., STRIANO, P., DILENA, R., FONTANA, E., ZARA, F., KURLEMANN, G., KLEPPER, J., THOENE, J. G., ARNDT, D. H., DECONINCK, N., SCHMITT-MECHELKE, T., MAIER, O., MUHLE, H., WICAL, B., FINETTI, C., BRUCKNER, R., PIETZ, J., GOLLA, G., JILLELLA, D., LINNET, K. M., CHARLES, P., MOOG, U., OIGLANE-SHLIK, E., MANTOVANI, J. F., PARK, K., DEPREZ, M., LEDERER, D., MARY, S., SCALAIS, E., SELIM, L., VAN COSTER, R., LAGAE, L., NIKANOROVA, M., HJALGRIM, H., KORENKE, G. C., TRIVISANO, M., SPECCHIO, N., CEULEMANS, B., DORN, T., HELBIG, K. L., HARDIES, K., STAMBERGER, H., DE JONGHE, P., WECKHUYSEN, S., LEMKE, J. R., KRAGELOH-MANN, I., HELBIG, I., KLUGER, G., LERCHE, H. & MOLLER, R. S. 2017. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain,* 140**,** 1316-1336.

WU, C., ZHANG, G., CHEN, L., KIM, S., YU, J., HU, G., CHEN, J., HUANG, Y., ZHENG, G. & HUANG, S. 2019. The Role of NLRP3 and IL-1β in Refractory Epilepsy Brain Injury. *Front Neurol,* 10**,** 1418.

WU, D., BACAJ, T., MORISHITA, W., GOSWAMI, D., ARENDT, K. L., XU, W., CHEN, L., MALENKA, R. C. & SUDHOF, T. C. 2017. Postsynaptic synaptotagmins mediate AMPA receptor exocytosis during LTP. *Nature,* 544**,** 316-321.

WU, H., LI, H., BAI, T., HAN, L., OU, J., XUN, G., ZHANG, Y., WANG, Y., DUAN, G., ZHAO, N., CHEN, B., DU, X., YAO, M., ZOU, X., ZHAO, J., HU, Z., EICHLER, E. E., GUO, H. & XIA, K. 2020. Phenotype-to-genotype approach reveals head-circumference-associated genes in an autism spectrum disorder cohort. *Clin Genet,* 97**,** 338-346.

XIE, Y., CASTRO-HERNÁNDEZ, R., SOKPOR, G., PHAM, L., NARAYANAN, R., ROSENBUSCH, J., STAIGER, J. F. & TUOC, T. 2019. RBM15 Modulates the Function of Chromatin Remodeling Factor BAF155 Through RNA Methylation in Developing Cortex. *Mol Neurobiol,* 56**,** 7305-7320.

XING, Z. K., ZHANG, L. Q., ZHANG, Y., SUN, X., SUN, X. L., YU, H. L., ZHENG, Y. W., HE, Z. X. & ZHU, X. J. 2020. DIP2B Interacts With α-Tubulin to Regulate Axon Outgrowth. *Front Cell Neurosci,* 14**,** 29.

YANG, X., QIAN, P., XU, X., LIU, X., WU, X., ZHANG, Y. & YANG, Z. 2017. GRIN2A mutations in epilepsy-aphasia spectrum disorders. *Brain Dev*.

YEO, G. & BURGE, C. B. 2004. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. *J Comput Biol,* 11**,** 377-94.

YERNA, X., SCHAKMAN, O., RATBI, I., KREIS, A., LEPANNETIER, S., DE CLIPPELE, M., ACHOURI, Y., TAJEDDINE, N., TISSIR, F., GUALDANI, R. & GAILLY, P. 2020. Role of the TRPC1 Channel in Hippocampal Long-Term Depression and in Spatial Memory Extinction. *Int J Mol Sci,* 21.

YILMAZ, S., GOKBEN, S., SERDAROGLU, G., ERASLAN, C., MANCINI, G. M., TEKIN, H. & TEKGUL, H. 2016. The expanding phenotypic spectrum of ARFGEF2 gene mutation: Cardiomyopathy and movement disorder. *Brain Dev,* 38**,** 124-7.

YOGARAJAH, M., MATARIN, M., VOLLMAR, C., THOMPSON, P. J., DUNCAN, J. S., SYMMS, M., MOORE, A. T., LIU, J., THOM, M., VAN HEYNINGEN, V. & SISODIYA, S. M. 2016. PAX6, brain structure and function in human adults: advanced MRI in aniridia. *Ann Clin Transl Neurol,* 3**,** 314-30.

YOO, Y., JUNG, J., LEE, Y. N., LEE, Y., CHO, H., NA, E., HONG, J., KIM, E., LEE, J. S., LEE, J. S., HONG, C., PARK, S. Y., WIE, J., MILLER, K., SHUR, N., CLOW, C., EBEL, R. S., DEBROSSE, S. D., HENDERSON, L. B., WILLAERT, R., CASTALDI, C., TIKHONOVA, I., BILGUVAR, K., MANE, S., KIM, K. J., HWANG, Y. S. & LEE, S. G. 2017. GABBR2 mutations determine phenotype in rett syndrome and epileptic encephalopathy. 82**,** 466-478.

YOON, W. J., WON, S. J., RYU, B. R. & GWAG, B. J. 2003. Blockade of ionotropic glutamate receptors produces neuronal apoptosis through the Bax-cytochrome C-caspase pathway: the causative role of Ca2+ deficiency. *J Neurochem,* 85**,** 525-33.

YUAN, H., HANSEN, K. B., ZHANG, J., PIERSON, T. M., MARKELLO, T. C., FAJARDO, K. V., HOLLOMAN, C. M., GOLAS, G., ADAMS, D. R., BOERKOEL, C. F., GAHL, W. A. & TRAYNELIS, S. F. 2014. Functional analysis of a de novo GRIN2A missense mutation associated with early-onset epileptic encephalopathy. *Nat Commun,* 5**,** 3251.

ZAGAGLIA, S., SELCH, C., NISEVIC, J. R., MEI, D., MICHALAK, Z., HERNANDEZ-HERNANDEZ, L., KRITHIKA, S., VEZYROGLOU, K., VARADKAR, S. M., PEPLER, A., BISKUP, S., LEÃO, M., GÄRTNER, J., MERKENSCHLAGER, A., JAKSCH, M., MØLLER, R. S., GARDELLA, E., KRISTIANSEN, B. S., HANSEN, L. K., VARI, M. S., HELBIG, K. L., DESAI, S., SMITH-HICKS, C. L., HINO-FUKUYO, N., TALVIK, T., LAUGESAAR, R., ILVES, P., ÕUNAP, K., KÖRBER, I., HARTLIEB, T., KUDERNATSCH, M., WINKLER, P., SCHIMMEL, M., HASSE, A., KNUF, M., HEINEMEYER, J., MAKOWSKI, C., GHEDIA, S., SUBRAMANIAN, G. M., STRIANO, P., THOMAS, R. H., MICALLEF, C., THOM, M., WERRING, D. J., KLUGER, G. J., CROSS, J. H., GUERRINI, R., BALESTRINI, S. &

SISODIYA, S. M. 2018. Neurologic phenotypes associated with COL4A1/2 mutations: Expanding the spectrum of disease. *Neurology,* 91**,** e2078-e2088.

ZAMAN, T., HELBIG, I., BOZOVIC, I. B., DEBROSSE, S. D., BERGQVIST, A. C., WALLIS, K., MEDNE, L., MAVER, A., PETERLIN, B. & HELBIG, K. L. 2018. Mutations in SCN3A cause early infantile epileptic encephalopathy. 83**,** 703-717.

ZAMARBIDE, M., MOSSA, A., MUÑOZ-LLANCAO, P., WILKINSON, M. K., POND, H. L., OAKS, A. W. & MANZINI, M. C. 2019. Male-Specific cAMP Signaling in the Hippocampus Controls Spatial Memory Deficits in a Mouse Model of Autism and Intellectual Disability. *Biol Psychiatry,* 85**,** 760-768.

ZEHAVI, Y., MANDEL, H., ZEHAVI, A., RASHID, M. A., STRAUSSBERG, R., JABUR, B., SHAAG, A., ELPELEG, O. & SPIEGEL, R. 2017. De novo GRIN1 mutations: An emerging cause of severe early infantile encephalopathy. *Eur J Med Genet,* 60**,** 317-320.

ZEREM, A., HAGINOYA, K., LEV, D., BLUMKIN, L., KIVITY, S., LINDER, I., SHOUBRIDGE, C., PALMER, E. E., FIELD, M., BOYLE, J., CHITAYAT, D., GAILLARD, W. D., KOSSOFF, E. H., WILLEMS, M., GENEVIEVE, D., TRAN-MAU-THEM, F., EPSTEIN, O., HEYMAN, E., DUGAN, S., MASUREL-PAULET, A., PITON, A., KLEEFSTRA, T., PFUNDT, R., SATO, R., TZSCHACH, A., MATSUMOTO, N., SAITSU, H., LESHINSKY-SILVER, E. & LERMAN-SAGIE, T. 2016. The molecular and phenotypic spectrum of IQSEC2-related epilepsy. *Epilepsia,* 57**,** 1858-1869.

ZHANG, X., LING, J., BARCIA, G., JING, L., WU, J., BARRY, B. J., MOCHIDA, G. H., HILL, R. S., WEIMER, J. M., STEIN, Q., PODURI, A., PARTLOW, J. N., VILLE, D., DULAC, O., YU, T. W., LAM, A. T., SERVATTALAB, S., RODRIGUEZ, J., BODDAERT, N., MUNNICH, A., COLLEAUX, L., ZON, L. I., SOLL, D., WALSH, C. A. & NABBOUT, R. 2014. Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. *Am J Hum Genet,* 94**,** 547-58.

ZHANG-JAMES, Y., VAUDEL, M., MJAAVATTEN, O., BERVEN, F. S., HAAVIK, J. & FARAONE, S. V. 2019. Effect of disease-associated SLC9A9 mutations on protein-protein interaction networks: implications for molecular mechanisms for ADHD and autism. *Atten Defic Hyperact Disord,* 11**,** 91-105.

ZHENG, F. 2017. TRPC Channels and Epilepsy. *Adv Exp Med Biol,* 976**,** 123-135.

ZIPPER, R., BAINE, S. D., GENIZI, J., MAOZ, H., LEVY, N. S. & LEVY, A. P. 2017. Developmental progression of intellectual disability, autism, and epilepsy in a child with an IQSEC2 gene mutation. 5**,** 1639-1643.

ZOU, F., MCWALTER, K., SCHMIDT, L., DECKER, A., PICKER, J. D., LINCOLN, S., SWEETSER, D. A., BRIERE, L. C., HARINI, C., MARSH, E., MEDNE, L., WANG, R. Y., LEYDIKER, K., MOWER, A., VISSER, G., CUPPEN, I., VAN GASSEN, K. L., VAN DER SMAGT, J., YOUSAF, A., TENNISON, M., SHANMUGHAM, A., BUTLER, E., RICHARD, G. & MCKNIGHT, D. 2017. Expanding the phenotypic spectrum of GABRG2 variants: a recurrent GABRG2 missense variant associated with a severe phenotype. *J Neurogenet,* 31**,** 30-36.

ZOU, L. B., YAMADA, K., SASA, M., NAKATA, Y. & NABESHIMA, T. 2000. Effects of sigma(1) receptor agonist SA4503 and neuroactive steroids on performance in a radial arm maze task in rats. *Neuropharmacology,* 39**,** 1617-27.

ZUBERI, S. M. & SYMONDS, J. D. 2015. Update on diagnosis and management of childhood epilepsies. *J Pediatr (Rio J),* 91**,** S67-77.

ABRAHAM, W. C. & WILLIAMS, J. M. 2003. Properties and mechanisms of LTP maintenance. *Neuroscientist,* 9**,** 463-74.

HERRING, B. E. & NICOLL, R. A. 2016. Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. *Annu Rev Physiol,* 78**,** 351-65.

LISMAN, J. 2017. Glutamatergic synapses are structurally and biochemically complex because of multiple plasticity processes: long-term potentiation, long-term depression, short-term potentiation and scaling. *Philos Trans R Soc Lond B Biol Sci,* 372.

NEVINS, A. K. & THURMOND, D. C. 2005. A direct interaction between Cdc42 and vesicle-associated membrane protein 2 regulates SNARE-dependent insulin exocytosis. *J Biol Chem,* 280**,** 1944-52.

RIZO, J., CHEN, X. & ARAC, D. 2006. Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends Cell Biol,* 16**,** 339-50.

WANG, Z., EDWARDS, J. G., RILEY, N., PROVANCE, D. W., JR., KARCHER, R., LI, X. D., DAVISON, I. G., IKEBE, M., MERCER, J. A., KAUER, J. A. & EHLERS, M. D. 2008. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell,* 135**,** 535-48.

WU, D., BACAJ, T., MORISHITA, W., GOSWAMI, D., ARENDT, K. L., XU, W., CHEN, L., MALENKA, R. C. & SUDHOF, T. C. 2017. Postsynaptic synaptotagmins mediate AMPA receptor exocytosis during LTP. *Nature,* 544**,** 316-321.