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# **Autoimmune Epilepsy in Childhood**

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## Summary

The role of immune system in seizures and epilepsy has been known for a long time. Inflammation is thought to play a role in many seizure disorders, such as in some severe childhood epilepsies and in fever and infection triggered seizures. Inflammation in these cases could be primary or secondary to the seizures themselves. Secondary inflammatory mechanisms are likely to be mediated "non-specifically" by the innate immune system.

The "specific" or adaptive immune system, which includes cell mediated and antibody mediated "humoral" mechanisms is now recognised to be important in some seizure disorders and recently the term "autoimmune epilepsy" has been used to imply involvement of the adaptive immune system, (particularly humoral) in the pathogenesis of epilepsy. Specific neuronal antibodies against cell surface proteins and receptors as well as some intracellular antigens are now well recognised in a proportion of adult patients with seizures. The seizures are often severe and not isolated, but rather accompanied by encephalopathy or other neurologic symptoms. Evidence of brain inflammation might be found in cerebrospinal fluid (pleocytosis or oligoclonal bands) or imaging (increased signal on magnetic resonance imaging). The best recognised disorders are limbic encephalitis (associated with antibodies against many neuronal antigens such as VGKC-complex proteins and GAD, and NMDAR encephalitis. However neuronal antibodies are also recognised in some adult patients with seizures and epilepsies in the absence of encephalopathy or other features. New antigenic targets are increasingly identified and at the time of reporting this includes: VGKC complex (LGI1, CASPR2 and contactin2 or TAG1), NMDAR, AMPAR, GABA<sub>B</sub>R, Glycine receptor and GAD. In disorders that are associated with antibodies to intracellular antigens (such as GAD), it is thought that cell mediated (cytotoxic) mechanisms are important rather than direct antigen-antibody interaction since the antigenic target is not readily exposed.

Most reports describing autoimmune epilepsies come from adult studies. At the time when I commenced my project in March 2009 there was no reports of neuronal antibodies in children, apart from NMDAR encephalitis. The available reports from adult studies suggested that the disorders associated with neuronal antibodies are potentially treatable with immunotherapy. While

immunotherapeutic agents (mainly corticosteroids and to a lesser extent intravenous immunoglobulin) have been used for a long time to treat some of the severe paediatric epilepsies such as West syndrome, Landau Kleffner and Lennox Gastaut syndromes, the mechanisms of their action are not clear and these agents are not given to target the immune system in this context.

My PhD project aimed to study and investigate neuronal antibodies in children with seizures in the context of encephalitis, or without encephalitis. This study hoped to help recognise children who may have ‘autoimmune seizures’. It was hoped that this could alter the treatment of patients with ‘autoimmune epilepsy’, from symptomatic treatment with conventional antiepileptic medications to immunotherapy that targets the underlying immune disorder.

The study was conducted at the Children Hospital at Westmead, which is one of the two tertiary children hospitals in Sydney. Based on the adult studies' findings, and the initial discovery of an index case, I first tested the acute serum of children presenting with unexplained encephalitis and severe seizures. I found serum VGKC-complex antibodies in four of the 10 patients tested. The clinical features of the affected children were similar to the adults described with VGKC encephalitis. I also used a paediatric control group to create a normal reference range for VGKC-complex antibodies in children. Secondly, I recruited a heterogeneous group of children with new onset seizures, and tested them for neuronal antibodies. 11 out of the 114 children (9.4%) tested positive for neuronal antibodies including VGKC complex, CASPR2 and NMDAR, mainly those with epilepsy of unknown cause (predominantly focal). This study also involved using the new ILAE classification system for epilepsy. Thirdly, I tested a separate miscellaneous group of patients who were suspected to have ‘autoimmune epilepsy’ through the clinical practise at the hospital. These patients were suspected to have autoimmune epilepsy due to the presence of CNS inflammation or associated autoimmune disorders. Neuronal antibodies were found in seven out of the 13 patients tested. I proposed clinical guidelines that may help in the identification of patients with autoimmune seizures.

The findings in this study are novel and have shed some light into the importance of neuronal antibodies in paediatric seizure disorders. While the pathogenic role of these antibodies remains a hot topic for debates and studies, we think that the presence of these antibodies can define a group of

patients where immune mechanisms are important, and where immunotherapy might improve the clinical outcome.

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## Statement on author's contribution

As the primary author (JS), I was the primary investigator and the main contributor to all phases of work leading to this thesis, except where acknowledgement has been made to the work of others. My contributions include:

1. Application for project approval by the hospital ethics committee.
2. Creation of information sheets and consent forms for both patients and parents.
3. Creation of databases for the different studies.
4. Selection of appropriate patients for the different studies and recruitment of patients for the prospective new onset seizure study.
5. Consenting of patients and their families (in person or by mail).
6. Collection of relevant data for patients included in the studies arising from this work; including patients' demographics, clinical information, investigations, follow up and outcome.
7. Arranging serum sample handling and dispatch to collaborators in UK for testing for appropriate antibodies (testing unavailable in Australia at the time of starting project).
8. Performing of data and results analysis including the use of relevant statistical methods when required.
9. Writing first drafts for papers and publications arising from this work, incorporating other authors' comments and revising manuscript as per journal suggestions when needed.
10. Writing the first draft of all chapters of this thesis, including tables and figures, and incorporating supervisor comments and suggestions when required.

Antibody assays were performed in Oxford, UK by Bethan Lang, Tanja Brenner and Sukhvir Wright.



# Presentations and Publications

## Presentations

1. World Congress Neurology (WCN), Austria, Vienna Sep 2013 (oral presentation: Neuronal antibodies in children with new onset seizures).
2. Australian and New Zealand Association of Neurologists (ANZAN) Annual Scientific Meeting, Australia, Hobart May 2011 (Oral presentation: VGKC complex antibody encephalitis in children with status epilepticus).
3. International Child Neurology Congress (ICNC), Egypt, Cairo, May 2010 (Poster presentation: VGKC encephalitis in a child, a case presentation).

## Publications

**Suleiman J**, Wright S, Gill D, Brilot F, Waters P, Peacock K, Procopis P, Nibber A, Vincent A, Dale RC, Lang B. Autoantibodies to neuronal antigens in children with new-onset seizures classified according to the revised ILAE organization of seizures and epilepsies. *Epilepsia*. 2013 Oct 23. [Epub ahead of print] PubMed PMID: 24151870.

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**Suleiman, J**, Brenner, T, Gill, D, Troedson, C, Sinclair, A, Brilot, F, Vincent, A, Lang, B, Dale, R. Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies. *Dev Med Child Neurol*. 2011 Nov; 53(11), 1058-60

**Suleiman, J**, Brenner, T, Gill, D, Brilot, F, Antony, J, Vincent, A, Lang, B, Dale, R. VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology*. 2011 April 5; 76(14), 1252-1255

## Abbreviations

|        |  |
|--------|--|
| Ab     | antibody   |
| Ach    | acetylcholine  |
| ACTH   | adrenocorticotrophic hormone                                   |
| ADH    | antidiuretic hormone   |
| ADHD   | attention deficit hyperactivity disorder                       |
| ADLTE  | autosomal dominant lateral temporal lobe epilepsy              |
| ADPEAF | autosomal dominant partial epilepsy with auditory features     |
| AED    | anti epileptic drug  |
| AERRPS | acute encephalitis with refractory repetitive partial seizures |
| AMPA   | alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid       |
| ANA    | antinuclear antibodies   |
| ANMT   | acquired neuromyotonia   |
| ARX    | aristaless-related homeobox gene                               |
| BFNIS  | benign familial neonatal-infantile seizures                    |
| BFNS   | benign familial neonatal seizures                              |
| CAE    | childhood absence epilepsy                                     |
| CASPR2 | contactin associated protein like 2                            |
| CDKL5  | cyclin-dependent kinase-like 5                                 |
| CMV    | cytomegalovirus  |
| CNS    | central nervous system   |
| CSE    | convulsive status epilepticus                                  |
| CSF    | cerebro-spinal fluid   |
| DESC   | devastating epileptic encephalopathy in school-aged children   |
| DG     | Deepak Gill  |
| DOB    | date of birth  |
| EBV    | Ebstein Barr virus   |
| EE     | epileptic encephalopathy                                       |
| EEG    | electroencephalogram   |
| EMG    | electromyography   |
| ENA    | extractable nuclear antigen                                    |
| FBDS   | faciobrachial dystonic seizures                                |
| FIRES  | Febrile-Infection Related Epilepsy Syndrome                    |
| FLAIR  | fluid attenuation inversion recovery                           |
| GABA   | gamma amino butyric acid                                       |
| GAD    | glutamic acid decarboxylase                                    |
| GEFS+  | generalised epilepsy with febrile seizures plus                |

|        |   |
|--------|---|
| GEPD   | generalised epilepsy with paroxysmal dyskinesia                 |
| GluR   | glutamate receptor  |
| GLUT1  | glucose transporter 1   |
| GlyR   | glycine receptor  |
| GTC    | generalised tonic clonic  |
| HEK    | human embryonic kidney cells                                    |
| HREC   | human research ethics committee                                 |
| HSV    | herpes simplex virus  |
| ICD    | international classifications of diseases                       |
| ICE    | international classification of epilepsy and epilepsy syndromes |
| ICES   | international classification of epileptic seizures              |
| IGE    | idiopathic generalised epilepsy                                 |
| ILAE   | international league against epilepsy                           |
| IS     | infantile spasms  |
| IVIG   | intravenous immunoglobulins                                     |
| JME    | juvenile myoclonic epilepsy                                     |
| JS     | Jehan Suleiman  |
| Kv     | voltage gated potassium channels                                |
| LE     | limbic encephalitis   |
| LGI1   | leucine rich glioma inactivated 1                               |
| MFC    | Morvan's fibrillary chorea                                      |
| MG     | myasthenia gravis   |
| MRI    | magnetic resonance imaging                                      |
| MRN    | medical record number   |
| MuSK   | muscle kinase   |
| NHMRC  | national health and medical research council                    |
| NMDA   | N-methyl-D-aspartate  |
| NMT    | neuromyotonia   |
| NORSE  | new onset refractory status epilepticus                         |
| NSAb   | neuronal surface antibody                                       |
| NSAS   | neuronal surface antibody syndrome                              |
| OCB    | oligoclonal bands   |
| PCDH19 | protocadherin 19  |
| PCR    | polymerase chain reaction                                       |
| PERM   | progressive encephalomyelitis with rigidity and myoclonus       |
| PICU   | paediatric intensive care unit                                  |
| PLE    | paraneoplastic limbic encephalitis                              |
| pM     | pico mole per litre   |

|        |  |
|--------|--|
| PNS    | peripheral nervous system                                |
| RCD    | Russell C Dale   |
| SCLC   | small cell lung carcinoma                                |
| SCN1A  | sodium channel neuronal type 1a subunit                  |
| SE     | status epilepticus                                       |
| SIADH  | syndrome of inappropriate antidiuretic hormone secretion |
| SLE    | systemic lupus erythematosus                             |
| SMEI   | severe myoclonic epilepsy of infancy                     |
| SPS    | stiff person syndrome                                    |
| SSA    | site specific assessment                                 |
| STXBP1 | syntaxin binding protein 1                               |
| SUDEP  | sudden death in epilepsy                                 |
| T1DM   | type 1 diabetes mellitus                                 |
| TLE    | temporal lobe epilepsy                                   |
| TTG    | tissue transglutaminase                                  |
| UKISS  | United Kingdom infantile spasms study                    |
| VGKC   | voltage gated potassium channels                         |

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# Chapter 1 Literature Review

In this chapter I present a summary of the various topics that are relevant to this study including seizures and epilepsy, and their classifications. I then introduce the concept of channelopathies in relation to epilepsy, both genetic and acquired. I discuss the important antibodies against various neuronal channels and proteins that are implicated in seizures and epilepsies, and present their relevant stories as they evolved. This is followed by the hypothesis and aims of my autoimmune epilepsy study in children.

## 1.1 Epilepsy

Epilepsy is one of the most prevalent neurological disorder in children affecting about 0.5 to 1.0% of children (Shinnar and Pellock, 2002). Wider ranges of prevalence rates for epilepsy in childhood have been reported from 0.15 to 12.1 %. The variation is likely due to geographical variations as well as study designs (Eriksson and Koivikko, 1997). The incidence of epilepsy is higher for children under 10 years and is highest for children less than one year (Camfield et al., 1996). Some of the childhood epilepsies remit during a predictable age group; however some epilepsies continue into adulthood. The majority of adults with epilepsy have onset in childhood (Shinnar and Pellock, 2002). Epilepsy causes significant morbidity and mortality in children. Co-morbidities are common in children with epilepsy including developmental disabilities, autism, attention deficit hyperactivity disorder (ADHD), depression and anxiety, migraine and accidental injuries (Pellock, 2004). Children suffering from epilepsy have a significantly increased mortality rate. The major causes of death are the underlying cause of the epilepsy itself, associated neurological compromise, injuries, status epilepticus and sudden death in epilepsy (SUDEP) (Shinnar and Pellock, 2002).

### 1.1.1 Definitions

An *Epileptic seizure* is defined as per the guidelines for epidemiologic studies, 1993 by the International League Against Epilepsy (ILAE) as “a clinical manifestation presumed to result from an abnormal and excessive discharge of a set of neurons in the brain”. The clinical manifestation consists

of “sudden and transitory abnormal phenomena, which may include alterations of consciousness, motor, sensory, autonomic, or psychic events, perceived by the patient or an observer” (Commission On et al., 1993).

*Epilepsy* is defined as a condition characterized by recurrent (two or more) epileptic seizures, unprovoked by any immediate identified cause (Commission On et al., 1993). Multiple seizures occurring in a 24 hour period and an episode of status epilepticus are considered single events. Febrile seizures and neonatal seizures (occurring in the first 30 days of life) are excluded and not considered a form of epilepsy (Commission On et al., 1993). In other words epilepsy is the occurrence of two or more unprovoked seizures more than 24 hours apart in a child older than 1 month. The diagnosis of epilepsy implies a persistent epileptogenic abnormality of the brain that is able to spontaneously generate paroxysmal activity (Engel, 2006).

### **1.1.2 Epilepsy Classifications**

Epilepsy has many types and different causes. Classification and terms vary considerably in different parts of the world. The International League Against Epilepsy (ILAE) Commission on Terminology has organised meetings of worldwide experts since 1964 to put in place a standard classification system that can be used internationally. The system defines seizure types according to their proposed origin in the brain and according to their clinical phenotype. It also classifies epilepsies according to their aetiology and according to the presence of recognisable constellations of features that may be defined as distinctive syndromes (Gastaut et al., 1964, Gastaut, 1970). The ILAE classification system is widely used and has been revised a few times since its implementation in 1969 to meet the ongoing developments in the understanding of epilepsy and its causes. In 1981 the commission on classification and terminology of the International League Against Epilepsy proposed a revised clinical and electroencephalographic classification of epileptic seizures, which is known as the International Classification of Epileptic Seizures (ICES) (Bancaud et al., 1981) . In 1989 there was a proposal for revised classification of epilepsies and epileptic syndromes, which is known as the International Classification of Epilepsy and Epileptic Syndromes (ICE) (Roger et al., 1989). ICES and

ICE have been widely used and accepted internationally. Further revisions were made in 2001 mainly defining some of the terminology used (Engel, 2001). A recent revision in 2010 proposed major changes in the terminology and concepts used for seizure and epilepsy classification (Berg et al., 2010). These different classifications are presented below.

### ***1.1.2.1 International Classification of Epileptic Seizures (ICES)-1981***

Epilepsy is classified according to seizure type, generalised or focal and each type is further sub classified according to seizure phenotype or “semiology”. The following is the ILAE classification of seizures proposed in 1981(ICES) (Bancaud et al., 1981).

#### 1) Partial (focal) seizures:

- a. Simple partial seizures (no impairment of consciousness)
- b. Complex partial seizures
  1. With impairment of consciousness at onset
  2. Simple partial onset followed by impairment of consciousness
- c. Partial seizures evolving to generalised tonic-clonic convulsions (GTC)
  1. Simple evolving to GTC
  2. Complex evolving to GTC

#### 2) Generalised seizures (convulsive or nonconvulsive):

- a. Absence seizures and atypical absence
- b. Myoclonic seizures
- c. Clonic seizures
- d. Tonic seizures
- e. Atonic seizures (Astatic)

#### 3) Unclassified epileptic seizures

This includes all seizures that cannot be classified because of inadequate or incomplete data.

This classification term also includes some neonatal seizures.

### **1.1.2.2 International Classification of Epilepsies and Epileptic Syndromes (ICE)-1989**

In 1989, a proposal for classification of epilepsies and epileptic syndromes was made. The International Classification of Epilepsies and Epileptic Syndromes (ICE) was created to supplement the International Classification of Epileptic Seizures (ICES)-1981 (Roger et al., 1989).

The ICE (1989) classified epilepsies according to their aetiology, as follows:

- Idiopathic epilepsy; when there is no underlying or primary cause for the epilepsy other than a proposed hereditary predisposition. Idiopathic is derived from the Greek “idios”, meaning self, own, or personal. Idiopathic epilepsies and syndromes are described as disorders “not preceded or occasioned by another,” in the Oxford English Dictionary (quoted from ICE 1989) (Roger et al., 1989). In idiopathic epilepsies there is no underlying structural brain abnormality or other neurologic signs or symptoms. Idiopathic epilepsies are age related (Engel, 2001).
- Symptomatic epilepsy; when there is a known or suspected underlying disorder of the central nervous system (CNS) (Roger et al., 1989) This includes structural brain abnormalities such as cortical malformation, brain tumours or other space occupying lesions, traumatic or ischaemic brain injuries, inflammatory conditions including meningitis and encephalitis and metabolic conditions.
- Cryptogenic epilepsy; cryptogenic refers to a disorder whose cause is hidden or occult. Cryptogenic epilepsies are presumed to be symptomatic, but the aetiology is not known (ICE 1989) (Roger et al., 1989).

An “*epilepsy syndrome*” is defined as a an epileptic disorder characterised by a cluster of signs and symptoms occurring together; these include items such as the type of seizure, aetiology, anatomy, precipitating factors, age of onset, severity, chronicity, diurnal and circadian cycling, and sometimes prognosis (Roger et al., 1989). An "epilepsy syndrome" does not necessarily have the same aetiologies or prognosis.

The following list is the international classification of epilepsy and epileptic syndromes as per ILAE International Classification of Epilepsies and Epileptic Syndromes (ICE)-1989:

1) Localisation related (partial, focal, local)

1.1 Idiopathic (with age related onset)

- a. Benign childhood epilepsy with centro-temporal spikes
- b. Childhood epilepsy with occipital paroxysms
- c. Primary reading epilepsy

1.2 Symptomatic temporal, frontal, parietal and occipital lobe epilepsies

1.3 Cryptogenic temporal, frontal, parietal and occipital lobe epilepsies

2) Generalised epilepsies and syndromes

2.1 Idiopathic

- a. Benign neonatal familial convulsions
- b. Benign neonatal convulsions
- c. Benign myoclonic epilepsy in infancy
- d. Childhood absence epilepsy
- e. Juvenile absence epilepsy
- f. Juvenile myoclonic epilepsy (impulsive petit mal)
- g. Epilepsy with grand mal (GTC) seizures on awakening
- h. Other generalised idiopathic epilepsies not defined above

2.2 Cryptogenic or symptomatic

- a. West syndrome
- b. Lennox-Gastaut syndrome
- c. Epilepsy with myoclonic-astatic seizures
- d. Epilepsy with myoclonic absences

2.3 Symptomatic

2.3.1 Non-specific aetiology

- a. Early myoclonic encephalopathy
- b. Early infantile epileptic encephalopathy with suppression burst

c. Other symptomatic generalised epilepsies not defined above

2.3.2 Specific syndromes

Epileptic seizures may complicate many disease states. Under this heading are included diseases in which seizures are a presenting or predominant feature

3) Epilepsies and syndromes undetermined whether focal or generalised

3.1 With both generalised and focal seizures

- a. Neonatal seizures
- b. Severe myoclonic epilepsy in infancy
- c. Epilepsy with continuous spike-waves
- d. Acquired epileptic aphasia (Landau-Kleffner- syndrome)
- e. Other undetermined epilepsies not defined above

3.2 Without unequivocal generalised or focal features.

This category includes cases with generalised tonic clonic (GTC) seizures in which clinical and EEG findings do not permit classification as clearly generalised or localization related.

Examples are many cases of sleep grand mal seizures which are considered to have equivocal generalised or focal features.

4) Special syndromes

4.1 Situation-related seizures

- a. Febrile convulsions
- b. Isolated seizures or isolated status epilepticus
- c. Seizures occurring only when there is an acute metabolic or toxic event due to factors such as alcohol, drugs, eclampsia, nonketotic hyperglycemia

Studies trying to use the classification found that a considerable number of cases fell into the nonspecific categories of ICES (cryptogenic, symptomatic), which limits the value of this epilepsy and syndrome classification (Manford et al., 1992, Eriksson and Koivikko, 1997).

### ***1.1.2.3 ILAE classification updates (Engel 2001 and 2006)***

The ILAE Classification of Epileptic Seizures (ICES) in 1981 and the ILAE Classification of Epilepsies and Epileptic Syndromes (ICE) in 1989 provided universal terminology that facilitated communication on clinical and research levels (Engel, 2001). In 1997 a Task Force on Classification and Terminology was appointed to revise the ILAE classification to accommodate the changes that occurred since the adoption of the classification used at the time, ICES and ICE. The Task Force divided itself into four working groups concerned with

1. Descriptive Terminology for Ictal Events,
2. Seizures,
3. Syndromes and Diseases, and
4. Impairment.

The Task Force decided that it was not possible to replace the current international classifications (ICE and ICES) with similar revised and updated classifications that would be universally accepted. Instead the Task Force focused on defining the terminology and proposed a diagnostic scheme for people with epileptic seizures and epilepsy that makes use of standardized terminology and concepts to describe individual patients (Engel, 2001) (for detailed reference see Tables 1 to 4 in Appendix 1). The Task Force also came up with a standardized glossary of descriptive terminology for ictal semiology (Blume et al., 2001), see Appendix 2.

The classification system was revisited again in 2006 and the ILAE commission commented then that the original classification of ICE and ICES remained acceptable and was not to be discarded unless a better classification was revised, although some modifications were anticipated (Engel, 2006).

### ***1.1.2.4 Latest proposed ILAE classification (Berg 2010)***

With major technologic understanding in the preceding decade, and improvement in the understanding of epilepsy (particularly the genetics of epilepsy), the need for a new classification system was recognised. In 2010 ILAE Commission on Classification and Terminology introduced a new version of revised terminology and concepts for classification of seizures and epilepsy syndromes

(Berg et al., 2010). The new report proposed changing a few existing terms and tried to use a simplified meaningful approach.

Seizures were broadly classified into focal, generalised and unknown;

1) Focal seizures; which were further classified according to the degree of impairment during seizures

- a. Without impairment of consciousness,
- b. With impairment of consciousness (dyscognitive- replaces the term complex partial),
- c. Evolving into bilateral convulsive seizure (replaces the term secondarily generalised).

2) Generalised Seizures

- a. Tonic- clonic
- b. Absence
  - Typical
  - Atypical
  - Absence with special features: myoclonic absence and eyelid myoclonia
- c. Myoclonic
  - Myoclonic
  - Myoclonic atonic
  - Myoclonic tonic
- d. Clonic
- e. Tonic
- f. Atonic

3) Unknown

- Epileptic spasms: which were previously called infantile spasms, however as they can continue beyond infancy the term 'epileptic spasms' was used instead. 'Epileptic spasms' were difficult to group into focal or generalised seizures, so were classified as unknown.

Previous classifications listed infantile spasms under generalised seizures.



- The new classification by Berg et al recommended new terminology in epilepsy aetiology classification to replace the existing terms symptomatic, idiopathic and cryptogenic. The following terms were recommended:
- Genetic (replaces Idiopathic) epilepsy is the direct result of a known or presumed genetic defect(s) in which seizures are the core symptom of the disorder. This may be supported by additional evidence.
- Structural/metabolic (replaces Symptomatic) epilepsy when there is a distinct structural or metabolic condition or disease that has been demonstrated to be associated with a substantially increased risk of developing epilepsy. Structural lesions can be acquired such as stroke, trauma and infection, or can be genetic such as Tuberous sclerosis.
- Unknown cause (replaces Cryptogenic) epilepsy where the underlying cause is not known as yet but it may be an unrecognised genetic, structural or metabolic cause (Berg et al., 2010, Berg and Scheffer, 2011).

The epilepsy syndromes were also revised and included the following subcategories (for more details see Table 5, Appendix 1):

- Electroclinical syndromes: This term replaced the term Epilepsy Syndromes in ICE 1989. An electroclinical syndrome is a complex of clinical features, signs and symptoms that together define a distinctive recognizable clinical disorder. They are identifiable based on typical age of onset, EEG characteristics, seizure type and other features. Examples include benign familial neonatal epilepsy, myoclonic epilepsy of infancy, Panayotopoulos syndrome and childhood absence epilepsy.
- Distinctive constellations: These are conditions that have distinctive features to recognise them as specific entities but are not exactly electroclinical syndromes; for example mesial temporal lobe epilepsy with hippocampal sclerosis, Rasmussen syndrome and Gelastic seizures.
- Epilepsies attributed to a known structural or metabolic cause such as malformation of cortical development, tumours, infection, trauma, stroke, etc.

- Epilepsies of unknown cause, which account for one third of all epilepsies. It was recommended that these epilepsies should be characterised according to the relevant features.
- Condition with epileptic seizures that are not traditionally diagnosed as epilepsy such as febrile seizures and benign neonatal seizures were kept in a separate group.

This new classification system was met with variable degrees of acceptance and criticism in the international community (Panayiotopoulos, 2011, Shorvon, 2011, Panayiotopoulos, 2012).

### **1.1.3 Status Epilepticus**

The term “status epilepticus” is used whenever a seizure persists for a sufficient length of time or is repeated frequently enough that recovery between attacks does not occur. Status epilepticus may be divided into partial (e.g., Jacksonian), or generalised (e.g., absence status or tonic-clonic status).

When very localized motor status occurs, it is referred to as *epilepsia partialis continua* (Roger et al., 1989).

Status epilepticus was further defined as a single epileptic seizure of more than 30 minute duration or a series of epileptic seizures during which function is not regained between ictal events over a period of at least 30 minutes (Commission On et al., 1993).

Status epilepticus (SE) is the most common neurological emergency in childhood and often requires intensive care treatment. Longer duration of seizures is associated with increased risk of morbidity and mortality (Chin et al., 2004).

SE is traditionally classified into convulsive and non-convulsive based on the presence of a motor component to the prolonged seizure. Though the term “convulsive” is considered a lay term it was endorsed in the new ILAE classification as it is used widely throughout medicine (Berg et al., 2010).

#### ***1.1.3.1 Status epilepticus classification***

A useful classification system for the causes of convulsive status epilepticus (CSE) in childhood based on the aetiology was proposed as follows (Gastaut, 1983, Chin et al., 2004, Chin et al., 2006):

1. “Prolonged febrile seizure” is convulsive status epilepticus (CSE) in a previously neurologically healthy child aged between 6 months and 5 years during a febrile (temperature >38°C) illness and in the absence of defined CNS infection.
2. “Acute symptomatic” is CSE in a previously neurologically normal child within a week of an identified acute neurological insult including bacterial meningitis, viral CNS infection, metabolic derangements, drug-related effects, head injury, hypoxia or anoxia, or cerebrovascular disease.
3. “Remote symptomatic” is CSE in the absence of an identified acute insult but with a history of a pre-existing CNS abnormality more than 1 week previously.
4. “Acute on remote symptomatic” is CSE within a week of an acute neurological insult or febrile illness in a child with previous neurological abnormality, including epilepsy. This category includes children with cerebral palsy with a febrile illness not of CNS origin, and children with hydrocephalus and obstructed ventriculoperitoneal shunts.
5. “Idiopathic epilepsy related” is CSE that is not symptomatic and occurs in children with a previous diagnosis of idiopathic epilepsy or when the episode of convulsive status epilepticus is the second unprovoked seizure that has led to a diagnosis of idiopathic epilepsy
6. “Cryptogenic epilepsy related” is CSE that is not symptomatic and occurs in children with a previous diagnosis of cryptogenic epilepsy or when the episode of convulsive status epilepticus is the second unprovoked seizure that has led to a diagnosis of cryptogenic epilepsy.
7. “Unclassified” is CSE that cannot be classified into any other group.

#### **1.1.4 Epileptic Encephalopathy**

The term “epileptic encephalopathy” (EE) is used to describe a condition in which the epileptiform abnormalities themselves are believed to contribute to the progressive disturbance in cerebral function (Engel, 2001). Epileptic encephalopathies refer to a group of conditions where the neurological function is impaired mainly due to epileptic activity which can be clinical seizures or prominent interictal epileptic activity (Dulac, 2001). Epileptic encephalopathies can occur at any age however it is most common and severe in infancy and early childhood (Berg et al., 2010) where they may have detrimental effects on the developing brain. The seizures in these conditions are severe and often

manifest as infantile or epileptic spasms. The electroencephalogram (EEG) in these conditions is usually very abnormal including burst suppression and high voltage chaotic pattern (hypsarrythmia and modified hypsarrythmia). It is thought that the epileptic activity itself leads to the severe cognitive and behavioural impairment beyond what is expected from the underlying condition itself. The cause of the encephalopathy might be the underlying disease, the epileptic process or a combination of both (Berg et al., 2010).

#### ***1.1.4.1 Epileptic encephalopathies in infants and young children***

Epileptic encephalopathies as listed by the ILAE Task Force report in 2001 (Engel, 2001) included the following:

- Early myoclonic encephalopathy (may contribute to progressive dysfunction)
- Ohtahara syndrome
- West syndrome
- Dravet syndrome (previously known as severe myoclonic epilepsy in infancy)
- Myoclonic status in non progressive encephalopathies
- Lennox–Gastaut syndrome
- Landau–Kleffner syndrome
- Epilepsy with continuous spike–waves during slow-wave sleep

Epileptic encephalopathy (EE) is associated with many conditions including genetic, structural and metabolic aetiologies. Idiopathic cases are also described where no cause can be found. Structural causes associated with EE include congenital disorders such as malformations of cortical development, as well as acquired disorders such as hypoxic ischaemic insults. Metabolic causes known to result in EE include biotinidase deficiency, pyridoxine/pyridoxal 5-phosphate dependency, and GLUT1 (glucose transporter 1) deficiency, as well as disorders of biopterin synthesis, creatine synthesis and folate metabolism (Pearl, 2009, Holland and Hallinan, 2010). It is important to recognise these disorders as many of them are potentially treatable. More genes are now identified and recognised in association with early onset epileptic encephalopathies and epileptic spasms,

particularly in children with normal brain imaging. Identified genetic causes of Epileptic encephalopathy include ARX (Aristaless-related homeobox), CDKL5 (Cyclin-Dependent Kinase-Like 5), STXBP1 (Syntaxin binding protein 1), SCN1A (Sodium channel neuronal type 1a subunit), PCDH19 (Protocadherin 19) and others (Holland and Hallinan, 2010, Mastrangelo and Leuzzi, 2012). In many cases of EE with epileptic spasms the cause is unknown despite extensive investigations. Out of 207 patients enrolled in United Kingdom Infantile Spasms Study (UKISS) 68 had no cause found (33%) (Osborne et al.).

Epileptic encephalopathy and epileptic spasms are usually difficult to treat and often do not respond to conventional AEDs. Steroids are considered to be the first line and are probably the most effective treatment used for epileptic encephalopathy and epileptic spasm (Go et al., 2012). Vigabatrin is also commonly used in treatment of EE and IS particularly in patients with tuberous sclerosis (Go et al., 2012). Different centres in the world tend to use different forms of steroids including intramuscular adrenocorticotrophic hormone (ACTH) and high dose oral prednisolone. High dose oral prednisolone at 40-60 mg/day as used in the 2004 United Kingdom Infantile Spasms Study (UKISS) has been shown to be as effective but less expensive than ACTH in the treatment of epileptic spasms (Kossoff et al., 2009). The mechanism of action of steroids in treating epileptic spasms is unclear although different theories are proposed. It is possible that steroids have both anticonvulsant as well as anti-inflammatory and immune modulation effects (Vezzani et al., 2011).

#### ***1.1.4.2 Epileptic encephalopathy in older children- FIRES and related conditions***

In older children a spectrum of epileptic syndromes and encephalopathies affecting previously normal children and leading to epileptic encephalopathy are recognised. These include Devastating Epileptic Encephalopathy in School-aged Children (DESC) reported by French authors (Mikaeloff et al., 2006), Acute Encephalitis with Refractory Repetitive Partial Seizures (AERRPS) reported by Japanese authors (Sakuma, 2009), Febrile-Infection Related Epilepsy Syndrome (FIRES) (van Baalen et al., 2009, van Baalen et al., 2010) and Fever-Induced Refractory Epileptic Encephalopathy in School-

aged children (FIRES) (Nabbout et al., 2010, Nabbout et al., 2011). A similar condition has also been described in adults with new-onset refractory status epilepticus (NORSE) (Wilder-Smith et al., 2005).

These terms seem to be used interchangeably to describe similar conditions, though the recent reports are increasingly using the term FIRES. The affected children are previously healthy, and usually school age children (rather than infants). They develop acute or subacute new onset refractory status epilepticus, often preceded by fever or infection and the fever usually persists during the acute phase. Encephalitis is often suspected due to the presence of fever, encephalopathy and status epilepticus. The term “pseudencephalitis” has been used in some of these cases for that reason (Mikaeloff et al., 2006). Extensive investigations usually fail to reveal any cause for the refractory epilepsy including infectious, metabolic, genetic or structural aetiologies (van Baalen et al., 2010, Nabbout et al., 2011, van Baalen et al., 2012). Cerebrospinal fluid (CSF) is normal or shows mild pleocytosis and MRI of the brain is normal or shows non-specific changes. The EEG shows high voltage slow background activity and focal seizures often with switching between hemispheres. The patients typically continue to have frequent seizures over many weeks. The seizures are usually focal with onset in the perisylvian or rolandic areas, sometimes with evolution to generalised seizures. Patients go on to develop chronic epilepsy without going through a latent phase and continue to have refractory focal seizures in the chronic phase.

The seizures are extremely resistant to multiple antiepileptic drugs. The only treatment reported to stop the seizures in the acute phase is barbiturate induced suppression coma. Ketogenic diet has been reported to be effective in some cases (Nabbout et al., 2010, Howell et al., 2012). The outcome is often poor and severe degrees of developmental and cognitive impairment are common (Ismail and Kossoff, 2011).

Sakuma proposed diagnostic criteria for this condition (AERRPS), which can be used for FIRES as they are probably the same condition (Sakuma, 2009).

Sakuma included the following **mandatory** findings:

- Acute onset of seizures or impairment of consciousness

- No preceding developmental delay or epilepsy
- Frequent and refractory focal seizures, necessitating long-term (2 weeks or more) anaesthesia with intravenous barbiturates or benzodiazepines to achieve burst-suppression coma on EEG
- Continuous switchover to refractory epilepsy without a latent period.

**Supportive findings included:**

- Preceding fever that may persist during the acute phase of illness
- CSF findings of mild pleocytosis or increased protein
- EEG findings of slow background acutely and multifocal spikes in the chronic phase with variable locations of seizures onset and frequent secondary generalisation
- Non-specific MRI abnormalities or occasional hyperintensities in mesial temporal lobe may be observed.
- Profound neurologic, cognitive and psychiatric impairment as well as occasional motor disability.

The cause for FIRES/AERRPS/DESC is unknown. An immune-mediated or inflammatory process has been hypothesized but is unproven (Sakuma, 2009, Sakuma et al., 2010, Specchio et al., 2010, Nabbout et al., 2011). Neuronal autoantibodies have been reported in sporadic cases with FIRES including anti glutamic acid decarboxylase (GAD) (Specchio et al., 2010), anti-GluR-epsilon-2 (antibody to glutamate receptor epsilon 2 subunit of N-methyl-D-aspartate receptor) (Sakuma et al., 2010), neuropil type anti neuronal antibodies (Milh et al., 2011), and voltage gated potassium channel (VGKC) Abs (Illingworth et al., 2011). However more typically patients with FIRES or DESC are negative for neuronal autoantibodies when tested (Kramer et al., 2011, Nabbout et al., 2011, Howell et al., 2012, van Baalen et al., 2012). Immunotherapy including intravenous immunoglobulin (IVIG) and steroids has been tried in a proportion of patients with FIRES. Out of 77 patients from multiple previous studies reported by Kramer et al 30 patients received IVIG and 29 received steroids (Kramer et al., 2011). Two patients out of the 30 treated with IVIG had benefit and no benefit was seen in those who received steroids. Four out of seven patients reported recently by Howell et al received immunotherapy in the form of IVIG, steroids and rituximab (one patient) and had no benefit (Howell

et al., 2012). The absence of neuronal antibodies and the negative response to immunotherapy do not strongly support an immune mediated process. Instead, the “neuronal excitation” and “genetic channelopathy” hypothesis has been proposed recently as a cause for some of these cases of FIRES (Ismail and Kossoff, 2011, Howell et al., 2012). However it might be difficult to make larger conclusions about the irrelevance of immune mechanisms in FIRES due to the retrospective nature, the incomplete/late treatment with immunotherapy and the incomplete or absent neuronal antibody testing in these studies.

### **1.1.5 Treatment of epilepsy**

Many antiepileptic drugs are available and are used widely to treat epileptic seizures. However about a third of seizures are resistant to anti epileptic medical treatment (Kwan and Brodie, 2000). Most of these drugs are considered “symptomatic” treatments, meaning that they stop seizures but do not modify the underlying process. It is thought that understanding the mechanisms involved in the generation of seizures and epilepsy should lead to novel treatments that modify the epileptic process (Vezzani et al., 2011). Other medical treatments used for severe and refractory seizures in children include steroids and intravenous immunoglobulins. The exact mechanisms of action of these agents in the treatment of severe epilepsy are not fully understood although anticonvulsant as well as immune modulatory effect is possible. Surgical treatment for medically refractory seizures with a structural lesion is increasingly performed and provides cure in many cases.

## **1.2 Channelopathies and epilepsy**

Voltage gated ion channels, such as potassium, calcium, sodium and chloride channels, and ligand gated ion channels, such as nicotinic acetylcholine (Ach), gamma amino butyric acid (GABA), glycine and ionotropic glutamate receptors are important in the regulation of neuronal excitability and synaptic transmission in central and peripheral nervous system (Collingridge et al., 2009).

Defects in the function of these transmembrane ion channels can lead to dysfunction in the central and peripheral nervous systems. Defects in these channels are referred to as “channelopathies”.



The role of channelopathies in acute or transient neurological disorders including epilepsy is increasingly recognised, usually in relation to genetic mutations (Weber and Lerche, 2008). The concept of acquired channelopathies is now well recognised (Berkovic et al., 2006, Helbig et al., 2008). Acquired causes of channelopathies include brain injury, stroke, tumours and infections. “Autoimmune channelopathies” are now recognised as an important cause of acquired channelopathies (Vincent et al., 2006). “Autoimmune channelopathies” are characterised by the presence of antibodies against neuronal channels (voltage or ligand gated) and various defects in the peripheral or central nervous system (or both). Autoimmune channelopathies are diagnosed by a simple laboratory test and are potentially treatable with immunotherapy that works on reducing the level of the pathogenic antibodies (Vincent et al., 2006).

### **1.2.1 Genetic Channelopathies**

Many mutations involving genes encoding for voltage and ligand gated channels proteins are described and the list is expanding. Mutations associated with monogenic disease affect almost all ion channel families: voltage-gated potassium, calcium and sodium channels, as well as ligand gated glycine, GABA and nicotinic acetylcholine receptors. Mutations have not been reported in association with the ionotropic glutamate receptors (Kullmann and Waxman, 2010).

Different seizure disorders and epilepsy syndromes are associated with different single gene mutations, although the same epilepsy disorder might be caused by more than one genetic mutation. For example childhood absence epilepsy (CAE) is associated with genetic mutations of calcium and chloride channels as well as GABA receptor. Likewise the same genetic mutation might be associated with more than one epilepsy syndrome; for example mutation of the GABA receptor GABRG2 is associated with CAE as well as febrile seizures and Dravet syndrome (see Table 1-1).

Genetic mutations involving the potassium channels are associated with benign familial neonatal seizures (BFNS) and generalised epilepsy with paroxysmal dyskinesia (GEPD) (Berkovic et al., 2006, Weber and Lerche, 2008). Leucine rich glioma inactivated-1 (LGI1) is part of the potassium channel complex. LGI1 gene mutation is associated with autosomal dominant lateral temporal lobe epilepsy

(ADLTE) or autosomal dominant partial epilepsy with auditory features (ADPEAF) (Morante-Redolat et al., 2002). Mutations involving genes encoding for calcium channels are associated with juvenile myoclonic epilepsy (JME) and childhood absence epilepsy (CAE). Genetic mutations of the sodium channels are associated with benign familial neonatal-infantile seizures (BFNIS), febrile seizures, severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome, and generalised epilepsy with febrile seizures plus (GEFS+) (Berkovic et al., 2006, Helbig et al., 2008, Weber and Lerche, 2008).

Many genetic "idiopathic" epilepsies however are not caused by a single-gene defect and complex inheritance is thought to play a role (Berkovic et al., 2006).

*Table 1-1 Genetic mutations of voltage and ligand gated channels*

*(A) Examples of genetic mutations of voltage gated channels or related proteins that are associated with epilepsy, adapted from (Berkovic et al., 2006, Helbig et al., 2008, Weber and Lerche, 2008).*

| Voltage gated channel type | Gene         | Disorder  |
|----------------------------|--------------|---|
| Potassium channels         | KCNQ2, KCNQ3 | Benign familial neonatal seizures (BFNS)                                  |
|                            | KCNMA1       | Generalised epilepsy and paroxysmal dyskinesia (GEPD)                     |
| Potassium channels related | LGII         | Autosomal-dominant partial epilepsy with auditory features                |
| Calcium channels           | CACNB4       | Juvenile myoclonic epilepsy (JME)   |
|                            | CACNA1H      | Childhood absence epilepsy (CAE)  |
| Sodium channels            | SCN1A        | GEFS+, febrile seizures, Dravet syndrome                                  |
|                            | SCN2A        | Benign familial neonatal-infantile seizures (BFNIS)                       |
|                            | SCN1B        | Generalised epilepsy with febrile seizures plus (GEFS+)                   |
| Chloride channel           | CLCN2        | Childhood absence epilepsy (CAE)<br>Idiopathic generalised epilepsy (IGE) |

(B) Examples of genetic mutations of ligand gated channels associated with epilepsy, adapted from (Berkovic et al., 2006, Helbig et al., 2008, Weber and Lerche, 2008).

| Ligand type             | Gene           | Disorder   |
|-------------------------|----------------|--|
| GABA receptor           | GABRG2         | Childhood absence epilepsy (CAE)<br>Dravet syndrome, febrile seizures, GEFS+ |
|                         | GABRA1         | Juvenile myoclonic epilepsy (JME)  |
|                         | GABRD          | Dravet syndrome, febrile seizures, GEFS+                                     |
|                         | GABRB3         | Childhood absence epilepsy (CAE)   |
| Acetyl choline receptor | CHRNA4, CHRNB2 | Autosomal dominant nocturnal frontal lobe epilepsy                           |

GEFS+ Generalised epilepsy with febrile seizures plus

### 1.3 Epilepsy and inflammation

It is thought that inflammatory processes within the brain might constitute a common and crucial mechanism in the pathophysiology of seizures and epilepsy (Vezzani et al., 2011). Several clinical facts support this hypothesis including the trigger of febrile seizures by proinflammatory agents (released by fever and infection), and the beneficial effect of steroids (which are anti-inflammatory agents) in some drug resistant epilepsies. Animal experimental models suggest that brain inflammation promotes neuronal hyper excitability and seizures. It has also been shown that seizures themselves initiate inflammation in the brain and cause damage of the blood brain barrier (Vezzani et al., 2011, Librizzi et al., 2012).

Autoimmune mechanisms are thought to play important roles in the aetiology of some seizures. Many childhood epilepsy syndromes that are refractory to conventional antiepileptic treatment and drugs may be responsive to some forms of immunotherapy such as steroids and IVIG (including West, Lennox-Gastaut and Landau-Kleffner syndromes). The mechanisms of action of immunotherapy in these disorders are not clear, though immune involvement has been proposed (Palace and Lang, 2000). Immune therapy may reduce refractory seizures by different mechanisms other than suppression of inflammation; immune therapeutic agents have significant anticonvulsant properties

either by direct effects on brain excitability and suppression of endogenous brain proconvulsants (Vezzani et al., 2011, Yu et al., 2013).

The association of epilepsy with autoimmune disorders such as systemic lupus erythematosus, vasculitis, coeliac disease and multiple sclerosis might be caused by immune system activation leading to brain inflammation causing epilepsy, although this hypothesis is not confirmed. Rasmussen encephalitis is an example of chronic brain inflammation leading to epilepsy. Autoantibodies against glutamate receptor type 3 (GluR3) were initially described in patients with Rasmussen encephalitis (Rogers et al., 1994), although this finding has not been subsequently reproduced (Watson et al., 2004). It is thought that T cell rather than antibody mediated immune mechanisms play a role in the chronic inflammation in Rasmussen syndrome.

The discovery of autoimmune limbic encephalitis and NMDAR encephalitis (see below) in association with specific neuronal autoantibodies provides further evidence that inflammatory and autoimmune mechanisms are important in the pathogenesis of seizures.

## **1.4 Neuronal antibodies associated with epilepsy and seizures, "autoimmune channelopathies"**

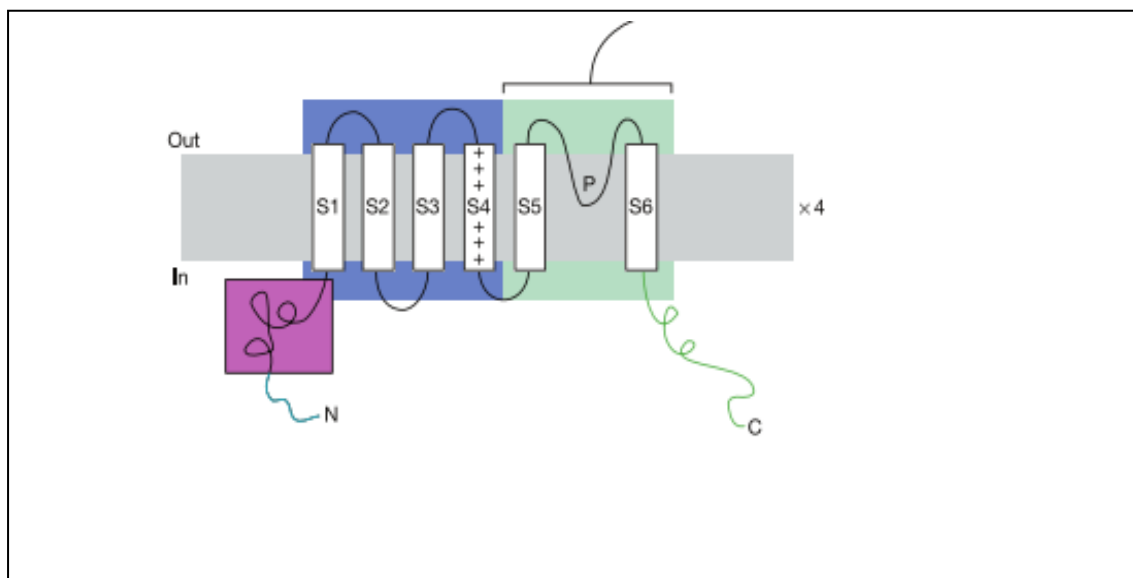
Autoimmune channelopathies associated with antibodies against voltage and ligand gated channels are increasingly recognised in the pathogenesis of many CNS disorders including seizures. "Immune mediated seizures" and "autoimmune epilepsies" are now described in association with antibodies to different neuronal surface proteins including receptors and synaptic proteins. The knowledge in this field has expanded rapidly in the last decade and new concepts and reports are published constantly. Below we discuss the different antibodies that are currently known to be associated with seizures and epilepsy. In the meantime the search continues and more antigenic targets are likely to be discovered.

### **1.4.1 VGKC antibodies and associated disorders**

Voltage-gated potassium ( $K^+$ ) channels (Kv) are transmembrane signalling proteins that are specific for potassium and have pores that allow the passage of millions of ions per second across the cellular

membrane. Their “gates” are sensitive to changes in voltage and they play a crucial role during action potentials in returning the depolarized cell to a resting state (Yellen, 2002). In humans, the Kv channels are diverse and divided into 12 subfamilies: Kv1-12 with 40 encoding genes (Gutman et al., 2005, Wulff et al., 2009).

Structurally the typical voltage-gated K channel (Kv) is an assembly of four identical transmembrane alpha subunits surrounding a central pore. Each alpha subunit has six transmembrane crossings (S1–S6). The narrowest part of the pore, the selectivity filter, is formed by a loop between S5 and S6; the voltage sensor includes the S4 region with its multiple positive charges, see Figure 1.1 (Yellen, 2002). A variety of accessory proteins are associated with some of the Kv tetramers which modulate the activity of the K<sup>+</sup> channels, including intracellular beta subunits (Gutman et al., 2005, Wulff et al., 2009).

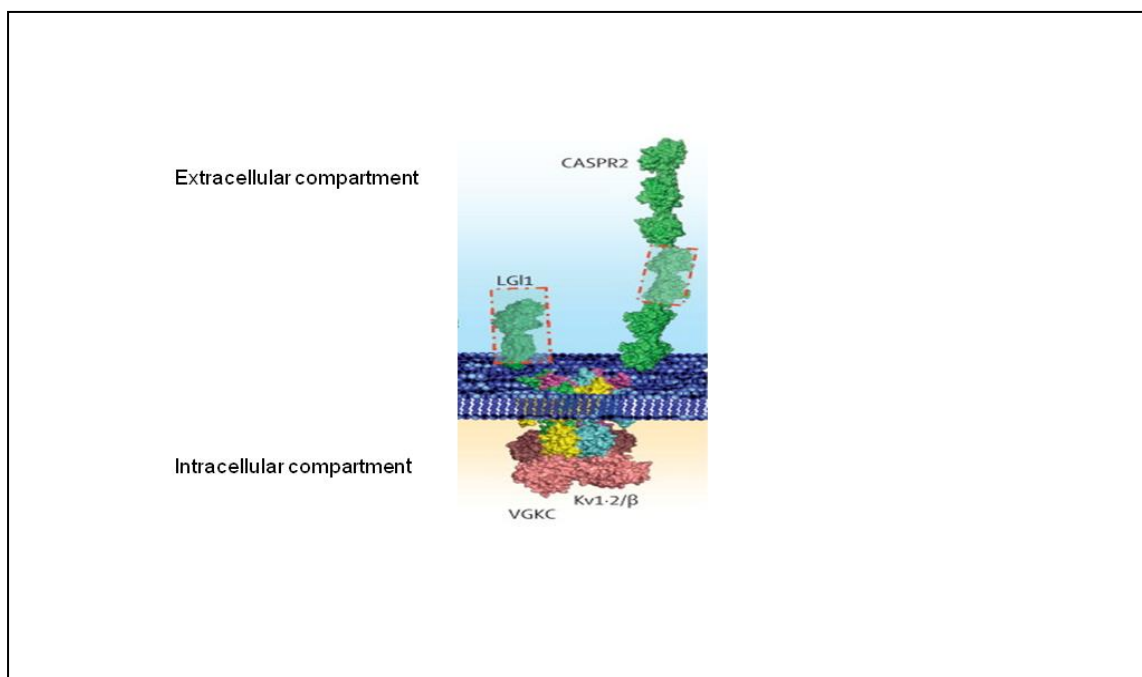


*Figure 1.1 The ultrastructure of a subunit of VGKC*

*(adapted from Yellen, 2002)*

The main function of the VGKC is to repolarise neuronal membrane following activation and depolarization by sodium and calcium channels as well as excitatory neurotransmitters including glutamate and acetylcholine. Dysfunction of VGKC leads to unopposed activation of the excitatory system, which leads to seizures (Singh et al., 1998).

Antibodies against voltage-gated potassium channel complex are one of the first recognised neuronal surface antibodies implicated in the pathogenesis of seizures and have been described in patients with peripheral and central nervous system disorders as described below. It should be noted that the actual antigenic target for VGKC-complex antibodies is often not the channel itself but other proteins that are part of the VGKC-complex, such as leucine -rich glioma inactivated 1(LGI1) and contactin-associated protein-like 2 (CASPR2), see Figure 1.2 (Vincent et al., 2011a). This will be discussed later.



*Figure 1.2 The structure of the VGKC and associated proteins*

*(adapted from Vincent et al 2011)*

#### ***1.4.1.1 Neuromyotonia, Isaac Syndrome and Morvan Syndrome associated with VGKC Abs***

Neuromyotonia (NMT) which also known as Isaac Syndrome is a rare neuromuscular disorder that results from hyperexcitability of the peripheral nerve axons leading to repetitive activation of single muscle fibres. NMT manifests as muscle cramps, fasciculation, stiffness and pain in addition to excessive sweating. Limb and trunk muscles are usually affected most although diffuse muscle involvement is also common. Muscle cramps occur spontaneously or can be triggered by muscle contraction. NMT has characteristic electromyographic (EMG) features which consist of repetitive motor unit or single fibre discharges that can be doublet, triplet or continuous and have a high intraburst frequency (Newsom-Davis and Mills, 1993, Newsom-Davis, 1997). There are hereditary cases of NMT associated with hereditary peripheral neuropathies however most cases are acquired. Acquired neuromyotonia (ANMT) is thought to be autoimmune in origin for a number of reasons as follows (Newsom-Davis and Mills, 1993). ANMT is described with other autoimmune disorders such as myasthenia gravis (MG) and with thymoma. Oligoclonal bands were found in cerebrospinal fluid (CSF) of some of the patients with ANMT and there was improvement of ANMT in patients who received plasma exchange (Newsom-Davis and Mills, 1993). Spontaneous remission occurred in patients with ANMT, which is a feature of some autoimmune disorders but not genetic disorders. In addition, ANMT can be seen in some patients with cancers as a paraneoplastic process. Antibodies against VGKC were found in 12 out of 12 patients with ANMT but not in controls and VGKC antibodies were proposed as the potential cause for ANMT (Hart et al., 1997).

Morvan Syndrome describes neuromyotonia that is accompanied by dysautonomia and central nervous system involvement. Morvan syndrome was first described in 1890 by Morvan and is also called Morvan's Fibrillary Chorea (MFC) or La chorée fibrillaire. Morvan syndrome is therefore characterised by neuromyotonia, hyperhidrosis and encephalopathy. Patients can have agitation, insomnia, confusion, hallucination, as well as tachycardia, pruritis and constipation. Morvan syndrome can be a paraneoplastic manifestation of thymoma and other tumours such as lung cancer. Morvan syndrome can also be associated with other autoimmune diseases (Lee et al., 1998). VGKC antibodies were identified in some patients with Morvan syndrome (Barber et al., 2000, Liguori et al.,

2001, Loukaides et al., 2012), although antibody negative cases are also described. Morvan syndrome is quite rare, often severe and the outcome is usually poor particularly in paraneoplastic cases.

#### ***1.4.1.2 Autoimmune limbic encephalitis and VGKC Abs***

Limbic encephalitis (LE) is a syndrome characterised by involvement of the limbic areas of the brain including the hippocampus, amygdala, hippocampal gyrus and cingulate gyrus. Patients have subacute memory disturbance, temporal lobe seizures, behavioural and personality changes as well as sleep disturbance (Bakheit et al., 1990, Gultekin et al., 2000, Buckley et al., 2001, Thieben et al., 2004). In the past LE was considered to be rare inflammatory and immune mediated paraneoplastic syndrome associated with tumours including occult small-cell carcinoma of the lung, thymoma, or testicular cancer. MRI and EEG in paraneoplastic limbic encephalitis (PLE) usually demonstrate abnormalities involving the temporal lobes. Patients with PLE usually have antibodies to intracellular neuronal antigens such as Hu, CV2 and Ma2, however these antibodies are thought to be markers of the paraneoplastic process rather than directly pathogenic autoantibodies. The diagnosis of PLE is confirmed by biopsy, which shows neuronal loss, reactive gliosis, and perivascular lymphocytic cuffing restricted to the limbic areas (Gultekin et al., 2000). No treatment is effective for PLE apart from early detection and treatment of the underlying neoplasm. PLE is unresponsive to immune therapy and often has different pathology compared to the immune responsive non paraneoplastic LE which is described below in more detail (Bien et al., 2012).

In 2001 Buckley et al described VGKC antibodies in two adult patients with reversible limbic encephalitis (LE) (Buckley et al., 2001). There were similarities noted between the PLE and the CNS symptoms of Morvan syndrome. This prompted Buckley et al to test two women with LE for VGKC Abs (which are found in some patients with Morvan syndrome), and these two women were positive for VGKC antibodies (Buckley et al., 2001). The first patient (47 years) had Myasthenia gravis and a thymoma that was previously excised. She was followed over the years and the onset of LE followed recurrence of her MG. LE symptoms responded to immunotherapy with plasma exchange, which was given to treat Myasthenia crisis. The VGKC antibodies in her serum were within normal range (<100



pM) until the onset of LE when they became elevated to 750 pM. The VGKC antibodies level fell with plasma exchange and this was associated with clinical improvement. The second patient (66 years) had no tumour and her serum had tested negative for the paraneoplastic antibodies. She improved spontaneously 15 months later. Retrospective testing of her acute serum for VGKC antibodies showed high titres (6000 pM) and her VGKC Ab subsequently spontaneously dropped after 18 months to 500 pM which also correlated with clinical improvement. Immunohistochemistry using the acute serum showed staining of the hippocampus, particularly the middle one-third of the molecular layer of the dentate gyrus. This pattern of staining was similar to that seen with anti-Kv1.2 antibody. This was the first report of LE associated with VGKC antibodies and it was proposed that this form of LE is potentially reversible or treatable, unlike the PLE (Buckley et al., 2001).

Vincent et al subsequently described 10 patients with VGKC Ab positive limbic encephalitis (LE) (nine male, one female; age range 44±79 years) (Vincent et al., 2004). The patients presented with 1-52 week histories of memory loss, confusion and seizures. Low plasma sodium concentrations, initially resistant to treatment, were present in eight out of the 10 patients and was thought to be secondary to inappropriate ADH (antidiuretic hormone) secretion. Brain MRI at onset showed signal changes in the medial temporal lobes in eight out of 10 cases. CSF was normal or only mildly abnormal and pleocytosis was uncommon. Five out of the ten patients had oligoclonal bands in the CSF with matching serum bands in four. EEGs showed non-specific changes including focal sharp waves mainly in the temporal regions, and slow waves that were mostly generalised but focal (temporal) in one patient. Paraneoplastic antibodies were negative, but VGKC-Ab titres were elevated and ranged from 450 to 5128 pM (neurological and healthy controls <100 pM). The VGKC antibodies were measured by radioimmunoassay which measures antibodies to the VGKC subtypes Kv1.1, 1.2 and 1.6 that bind <sup>125</sup>I-labelled dendrotoxin. Formal neuropsychology testing showed severe and global impairment of memory, with sparing of general intellect in all but two patients, and of nominal functions in all but one. Variable regimes of steroids, plasma exchange and intravenous immunoglobulin were associated with variable falls in serum VGKC-Abs to values between 2 and 88% of the initial values, together with marked improvement of neuropsychological functioning in six

patients, slight improvement in three and none in one. The improvement in neuropsychological functioning in seven patients correlated broadly with the fall in antibodies. However, varying degrees of cerebral atrophy particularly of mesial temporal structures and residual cognitive impairment were common (Vincent et al., 2004).

Around the same time Thieben et al 2004 described seven patients with encephalitis associated with autoantibodies to voltage-gated potassium channels (VGKCs) who were aged 44-73 years (2 females and 5 males) (Thieben et al., 2004) . Six out of the seven patients had cognitive impairment. Seizures of temporal lobe-onset 'complex partial' type were documented in six patients, and all seven patients had EEG abnormalities. Brain MRI showed mesial temporal lobe abnormalities in all seven patients. Four patients had additional neurologic autoantibody markers (muscle acetylcholine receptor, voltage gated calcium channel, or GAD), and two had anti thyroperoxidase antibodies. Two patients had a history of cancer; one had active prostate adenocarcinoma, and the second had a remote history of tongue carcinoma. One patient improved spontaneously and six were treated with IV methylprednisolone of whom three improved remarkably with treatment. At follow-up evaluation, one patient had no cognitive deficits, four had mild persistent short-term memory dysfunction, and two had persistent disabling behavioural deficits (Thieben et al., 2004).

Autoimmune LE became an increasingly recognised syndrome that is potentially reversible and treatable. VGKC- Abs are the most common neuronal antibodies associated in LE although LE has also been reported in association with other neuronal antibodies including, GABA<sub>B</sub>R and alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) antibodies, see below (Lai et al., 2009, Lancaster et al., 2010, Boronat et al., 2011). These antibodies are targeted against extracellular surface antigens, compared to onconeural antibodies targeted against intracellular antigens in paraneoplastic LE. The nature of these antibodies might explain the immunopathologic differences between these two conditions. Autoimmune non paraneoplastic LE however is also described in association with antibodies against GAD which is an intracellular antigen (Malter et al., 2010). The pathogenic role of these antibodies is discussed later.

LE is recognised as a potential cause of temporal lobe epilepsy (TLE) and mesial temporal sclerosis (MTS) in adults (Bien et al., 2007a, Bien et al., 2007b). Patients with LE develop unilateral or bilateral temporal lobe swelling and high signal on T2 and FLAIR (fluid attenuation inversion recovery) MRI sequences. This is often followed by atrophy and sclerosis of the mesial temporal lobes on serial MRI scans which may lead to the development of TLE in the subsequent years (Urbach et al., 2006, Bien et al., 2007b).

Bien et al proposed diagnostic criteria for both paraneoplastic and autoimmune LE in adults. The criteria included the presence of a recent onset (less than 5 years) limbic syndrome with at least one of the following clinical features: memory disturbance, temporal lobe seizures or affective and mood alteration. In addition there needed to be one of four diagnostic features including the presence of tumour (for PLE), the presence of onconeural or neuronal surface antibodies, MRI abnormality in temporal lobes, or lymphocytic inflammatory cells in the temporal lobes on histopathology (Bien et al., 2007a).

Many cases of VGKC-complex Ab associated LE have been reported since its first detailed description in 2004 by Vincent et al. The syndrome of VGKC Ab associated LE is now a well-defined syndrome that is generally thought to have a favourable outcome.

The VGKC antibody assay was based on immunoprecipitation of protein complexes that contain Kv1.1, Kv1.2, and Kv1.6 subunits of the VGKC which bind to the <sup>125</sup>I labelled dendrotoxin. In 2010 the VGKC antibodies in adults were found to bind other antigens and proteins closely related to the potassium channel rather than the potassium channel subunits themselves. The proteins identified were leucine -rich glioma inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2) and contactin 2 (Irani et al., 2010a, Lai et al., 2010). Lai et al showed that 57/57 patients with LE who previously tested positive for the VGKC antibodies had antibodies against LGI1 (100%). LGI1 was therefore proposed to be the autoantigen associated with limbic encephalitis previously associated with antibodies against voltage-gated potassium channels (Lai et al., 2010). Irani et al studied 96 sera that previously tested positive to VGKC-Abs. Binding to LGI1 transfected human embryonic kidney

(HEK293) cells was demonstrated in 55 of the 96 VGKC-antibody positive sera (57%). Binding to the surface of HEK293 cells transfected with CASPR2 was found in 19 out of the 96 VGKC-antibody positive sera (20%). Five of these 96 patients had antibodies to contactin-2 and three had antibodies to Kv1 subunits (Irani et al., 2010a). CASPR 2 Abs have generally been associated with peripheral nervous system disease whereas LGI1 Abs have been associated with LE (Lai et al., 2010, Irani et al., 2011b). The term "VGKC-complex Abs" is now used to reflect the variety of proteins that bind to the previously detected VGKC antibodies, other than the channel itself. It is likely that other proteins in the VGKC complex are involved in antibody binding, as described later in this thesis.

#### ***1.4.1.3 Seizures and epilepsy associated with VGKC Abs***

As described above VGKC-complex Abs were identified first in patients with neuromyotonia and Morvan syndrome, then in patients with LE presenting with memory disturbance, confusion and seizures.

Further reports suggested that VGKC-Abs are present in some patients with epilepsy without a preceding LE. Increased titres of VGKC antibodies (>100 pM) were detected in 16 of 139 (11%) patients with complex epilepsy but only 1 control (0.5%) (McKnight et al., 2005). Half of these VGKC Ab positive patients described by McKnight et al had short duration of their epilepsy (1-16 week) and half had long duration (more than 6 years). Ten out of the 16 VGKC Ab positive patients had associated immune-mediated disorders such as systemic lupus erythematosus (SLE), antiphospholipid syndrome and Hashimoto encephalopathy. MRI was normal in most patients but three of the positive patients had unilateral or bilateral hippocampal high signal. Three patients improved spontaneously and six received immunotherapy for their associated neurological conditions, five of which were reported to have some improvement (McKnight et al., 2005).

In a separate study, six out of 106 women with long standing intractable epilepsy (Majoie et al., 2006) were positive for VGKC-Abs with a range of 118-1406 pM (normal <100). The sera for VGKC- Ab testing were obtained many years after the onset of seizures which was in childhood or adolescence in five out of the six positive patients. The authors suggested that antibody titres might have been higher

if tested at the onset of seizures. None of these six patients had MRI abnormalities unlike what has been described in adults with VGKC- Ab associated limbic encephalitis (Majoie et al., 2006).

VGKC antibodies were found in an adult case with new onset refractory epilepsy that responded to immunotherapy (Barajas et al., 2010). In this case the seizures were of unusual semiology; described as unilateral twitches of shoulder and occasionally the ipsilateral face and occurred up to 30 per minute. MRI showed high signal in the mesiotemporal structures and the patient developed behavioural and personality changes. It is likely that the seizures preceded the onset of LE in this case, and the seizure semiology is reminiscent of faciobrachial dystonic seizures as described below (Irani et al., 2011b).

In a recent study of cohorts of adults with new onset and with established epilepsy VGKC complex antibodies were present in 5% of the patients (Brenner et al., 2013).

#### **1.4.2 LGI1 and CASPR2 antibodies**

Leucine -rich glioma inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2) and contactin 2 are proteins that are closely associated with the VGKC and are now recognised as part of the so called VGKC-complex (see Figure 1.2).

##### **1.4.2.1 LGI1 antibodies**

After the description of leucine rich glioma inactivated-1 (LGI1), a neuronal secreted protein as a major autoantigen for VGKC-complex Abs, attention has subsequently focused on LGI1. LGI1 had been identified as a subunit of pre-synaptic Kv1 voltage gated potassium channels (Schulte et al., 2006). Mutations in LGI1 are linked to autosomal dominant partial epilepsy with auditory features (ADPEAF), which is also known as autosomal dominant lateral temporal lobe epilepsy (ADLTE) (Morante-Redolat et al., 2002). It has been proposed that LGI1 is an anti-epileptogenic secreted protein that connects pre and postsynaptic protein complexes and regulates synaptic transmission (Fukata et al., 2010).

LGI1 antibodies are now recognised in adults to be associated predominantly with limbic encephalitis with seizures and cognitive impairment. Distinctive seizure semiology has been described in association with LGI1 antibodies and sometimes precedes the onset of cognitive impairment or encephalopathy. These were called faciobrachial dystonic seizures (FBDS) (Irani et al., 2008, Irani et al., 2011b). Irani et al described 29 adult patients with these seizures. The episodes of FBDS were frequent, brief dystonic seizures that predominantly affected the arm and ipsilateral face. They occurred up to 50 per day and some were associated with loss of consciousness. These seizures preceded the onset of LE in most patients. Investigation in the pre LE stage showed normal sodium in 13 patients tested, normal MRI in 9 patients performed and ictal epileptiform activity in 7 out of the 29 patients in the frontotemporal region. These seizures deteriorated during the evolution to LE in 26 patients after a mean of 36 days. LE was similar to what had been previously described in association with VGKC-complex Ab with amnesia, confusion and sleep disturbance. VGKC-complex Abs were detected by radioimmunoprecipitation in 24 patients and LGI1 Abs were found in 22 of them (2 of them also had Abs against CASPR2, and one also had Abs against contactin-2) whereas in two patients the target antigen was not identified, two patients tested negative for the VGKC-complex antibodies however one of them was positive for LGI1 antibodies by the cell-based assay. Investigations of the 26 patients after LE onset showed hyponatraemia in 23 (88%). Interictal EEG showed abnormalities including diffuse mild slowing in nine, bilateral frontotemporal slowing in six, and temporal sharp waves in two. In nine patients, no EEG abnormalities were found. In 14 out of the 26 patients (54%) MRI showed abnormalities during the LE stage including bilateral or unilateral medial temporal lobe high signal in 13 and right caudate and putamen high signal in one patient. Follow up MRI showed atrophy in the high signal areas in five patients including the one with the caudate abnormality. MRI was normal in 12 out of the 26 patients (46%). These distinctive seizures were resistant to multiple AEDs but immune responsive, and the authors emphasized the importance of their recognition (Irani et al., 2011b).

Similar episodes of seizures associated with LGI1 antibodies were described by Andrade et al although they were called "tonic seizures" (Andrade et al., 2011b). Three adult females were

described with abnormal movements of face, shoulders, arms and legs occurring up to 100 per day with preserved consciousness. These movements were associated with electrodecrement events on EEG and were thought to be tonic seizures. The three patients had high LGI1 levels however it was not clear if they had coexisting LE and whether these seizure episodes preceded their LE. Debates about the origin of these seizures and whether they actually represent extra pyramidal movements rather than epileptic tonic seizures were raised (Striano et al., 2011). Moreover as these movements were thought to be similar to the previously described FBDS, and disagreement about terminology were discussed between the authors (Andrade et al., 2011a, Irani et al., 2011c).

#### **1.4.2.2 CASPR2 antibodies**

Contactin-associated protein-like 2 (CASPR2) is a membrane protein with a large extracellular component and is closely complexed with the VGKCs (see Figure 1.2) (Vincent et al., 2011a). CASPR2 has a role in neuronal growth and synaptogenesis. Mutation in the CASPR2 gene has been found in patients with focal epilepsy and cortical malformations (Strauss et al., 2006).

CASPR2 antibodies were found in patients with Morvan syndrome in association with thymoma (Vincent, 2009, Vincent and Irani, 2010). CASPR2 has been identified as one of the auto antigens for VGKC-complex antibodies and is associated with disorders previously attributed to VGKC Abs including encephalitis, peripheral nerve dysfunction, or a combination of both (Morvan syndrome) (Lancaster et al., 2011a). CASPR2 antibodies are found in patients with LE and seizures although less commonly than LGI1 antibodies. More often patients with CASPR2 antibodies have Morvan's phenotype with neuromyotonia, confusion, amnesia, insomnia and autonomic dysfunction or neuromyotonia alone. By contrast, neuromyotonia is less common in patients with LGI1 Abs (Irani et al., 2010a). 19 out of 96 patients who previously tested positive for VGKC Abs were positive for CASPR2 Abs (Irani et al., 2010a). Three out of the 19 patients with positive CASPR2 Abs had Morvan's syndrome, seven had neuromyotonia, seven had LE and two had epilepsy alone (Irani et al., 2010a). Lancaster et al described eight patients with CASPR2 Abs; five had CNS involvement (encephalitis, seizures) as well as peripheral nervous system (PNS) involvement (neuromyotonia,

neuropathy), two had pure CNS syndrome and one had neuromyotonia alone (Lancaster et al., 2011a). Patients with CASPR2 Abs can have associated tumours particularly thymomas and small lung cancer (Vincent et al., 2011a). While most patients with the distinctive FBDS had LGI1 antibodies, two had CASPR2 antibodies in addition to LGI1 antibodies (Irani et al., 2011b). Seizures are usually not a prominent feature of patients with CASPR2 Abs.

### **1.4.3 VGKC-complex Abs in Children**

At the time of commencing this study in 2009 there had been no significant reports of VGKC Abs or other neuronal antibodies in children. However, in the last few years there have been a few reports examining VGKC-complex antibodies in children with epilepsy and seizures, which are discussed below for completeness. Limbic encephalitis associated with antibodies to potassium channels leading to bilateral hippocampal sclerosis was reported in a prepubertal girl (Kroll-Seeger et al., 2009). The girl presented with a subacute syndrome comprising memory impairment, affective disturbances, and refractory temporal lobe seizures, similar to adults with LE. In 2011, Limbic encephalitis was also described in 10 children of whom four were positive for VGKC Abs (Haberlandt et al., 2011). In this study, serum values for VGKC Abs were defined as normal (<100 pmol/l), low positive (100–150 pmol/l), positive (150–400 pmol/l) and high positive (>400 pmol/l) (Haberlandt et al., 2011). Two of the VGKC Abs positive patients were also positive for GAD antibodies, and two patients tested positive for GAD antibodies alone. These patients had poor outcomes, despite immunotherapy which was given relatively late in the course of their illness (median 4 months) (Haberlandt et al., 2011).

As part of the publications arising from this thesis; we described four patients with status epilepticus in the context of unexplained encephalitis who had antibodies against the VGKC- complex. In these patients, antibodies were not targeted against LGI1 or CASPR2 (Suleiman et al., 2011a), ( see Chapter 2). Our four patients had poor outcome including cognitive impairment, psychiatric disorder and ongoing epilepsy (mostly TLE). None of these patients received early or aggressive immunotherapy (Suleiman et al., 2011a).

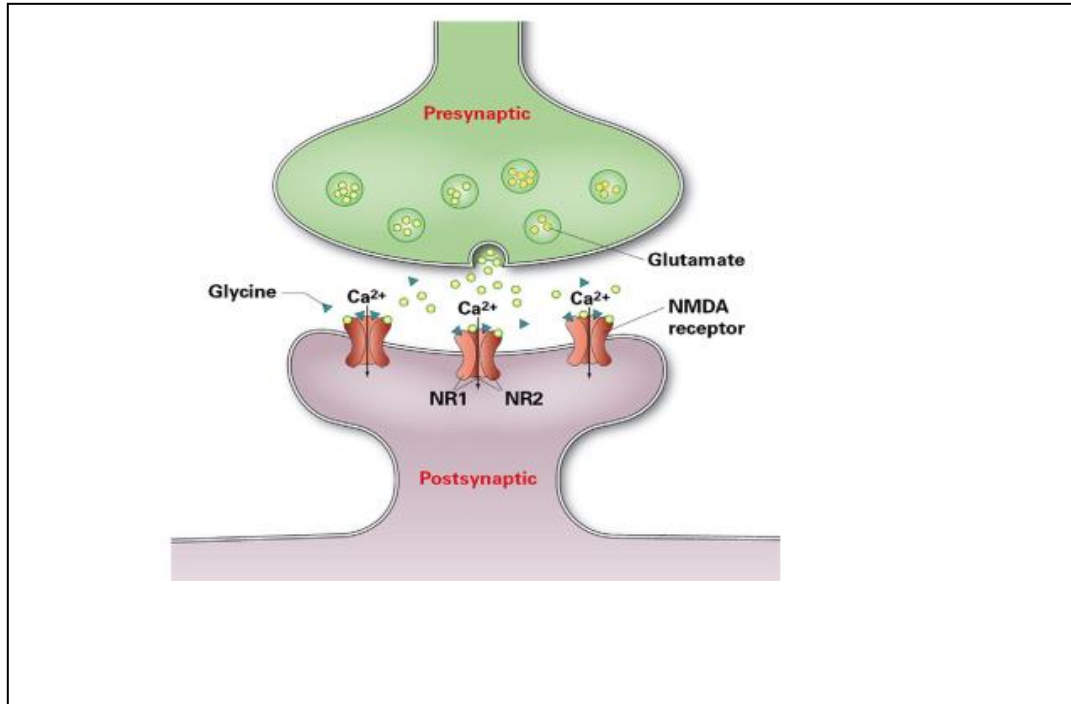


In a lab based study reporting the clinical phenotypes of children with positive VGKC-complex antibodies, three out of 12 children with positive VGKC-complex antibodies had seizures including one with limbic encephalitis and two with symptomatic generalised epilepsy (Dhamija et al., 2011). The other children with positive VGKC Abs in this study had variable neurological manifestations including developmental regression and movement disorders (Dhamija et al., 2011). We also reported VGKC-complex antibodies in a case of epileptic encephalopathy and epileptic spasms, which were refractory to AED but responsive to steroids (Suleiman et al., 2011b). This was the first report of specific autoimmunity in children with epileptic encephalopathy. Other paediatric reports include a previously normal 4 year old boy who presented with febrile illness, encephalopathy and multifocal seizures followed by status epilepticus and was thought to have FIRES (Fever-induced refractory epileptic encephalopathy in school-age children) (Illingworth et al., 2011). This child was positive to VGKC-complex antibodies and responded to immunotherapy in the form of steroids and immunoglobulin. We suggest that this case was more reminiscent of limbic encephalitis than FIRES as the patient responded to immune therapy, and had a good outcome.

There are no reports in children who are positive for LGI1 or CASPR2 antibodies at the time of writing. The reported children positive to VGKC-complex antibodies that were also tested for LGI1 and CASPR2 were negative (Dhamija et al., 2011, Illingworth et al., 2011, Suleiman et al., 2011a, Suleiman et al., 2011b) . It is likely that in children the antibodies that bind VGKC-complex are targeted against other antigens that are yet to be identified.

#### **1.4.4 NMDAR antibodies**

The N-methyl-D-aspartate (NMDA) receptors are ligand-gated channels that have important roles in synaptic transmission and plasticity. NMDAR mediates the post synaptic excitatory effects of glutamate (Bliss and Collingridge, 1993, Lynch et al., 1994). The NMDA receptors are heteromers of NR1 subunits that bind glycine, and NR2 (A, B, C, or D) subunits that bind glutamate (Dalmau et al., 2008), see Figure 1.3.



*Figure 1.3 NMDA type glutamate receptor at the post-synaptic region*

*(adapted from Wandinger et al., 2011)*

#### **1.4.4.1 NMDAR Ab encephalitis**

A form of severe and potentially lethal paraneoplastic encephalitis was recognised and reported in 2005 (Vitaliani et al., 2005). Affected patients were young women who developed prominent psychiatric symptoms, seizures, memory deficits, and decreased level of consciousness often requiring ventilatory support. They had associated ovarian teratomas. Unlike other paraneoplastic encephalitis syndromes where patients have onconeural antibodies these patients had antibodies to unknown antigens predominantly expressed in the cell membrane of hippocampal neurons (also referred to as neuropil antigens) that were detected in the sera and CSF of these patients (Vitaliani et al., 2005). The target autoantigens were later identified to be NR1 and NR2 subunits of the N-methyl-D-aspartate receptor (NMDAR) (Dalmau et al., 2007). Dalmau et al in 2007 reported 12 female patients with severe form of paraneoplastic encephalitis similar to that reported by Vitaliani et al. The affected women were of young age median 27 and range 14-44 years. They all had teratomas (11 ovarian and one mediastinal). All the 12 women had psychiatric symptoms including personality and behavioural change, agitation, or paranoid thoughts and six of them were evaluated initially by a

psychiatrist. Eleven patients developed seizures, which were generalised or complex partial. Nine patients had reduced level of consciousness requiring mechanical ventilation. Seven patients had abnormal movements including choreoathetosis, myoclonic movements, dystonia, dyskinesias in face and arms, rhythmic contractions of the abdominal wall, opisthotonic-like postures, and catatonic-like episodes. Hyperthermia was present in eight patients, and other autonomic disturbance including tachycardia, tachypnea, diaphoresis, or hypertension, and hypotension were seen in five patients (Dalmau et al., 2007). EEG showed generalised slowing with occasional epileptiform activity. MRI brain was often normal, although hyperintensities involving mesial temporal lobes, frontal and parietal lobes, cerebellum, and subtle meningeal enhancement were also described. CSF showed pleocytosis in all patients, but all patients were negative for viral, bacterial and autoimmune investigations including VGKC antibodies. The 12 patients were treated with tumour resection and immunotherapy (seven cases), surgery alone (three cases) or immunotherapy alone (two cases). Seven patients made a complete recovery, two had a partial recovery and three died. Patients who received both surgery and immunotherapy had generally better outcomes (Dalmau et al., 2007). These patients had neuropil antibodies which were found to be directed against the NR1 and NR2 subunits of the NMDAR and these antigens were also expressed by the associated tumours. It was suggested that this severe form of paraneoplastic encephalitis is antibody mediated and potentially reversible. The main epitope targeted by the antibodies was found to be in the extracellular N-terminal domain of the NR1 subunit of the NMDAR heteromers (Dalmau et al., 2008).

More patients were identified with NMDAR-Ab encephalitis and the syndrome became well characterized. Men are affected to a lesser extent than women and they can have associated tumours including testicular teratoma or lung cancer (Dalmau et al., 2008). Patients had a better outcome when recognised and treated early with tumour removal and immunotherapy. Non paraneoplastic cases affecting both males and females are also increasingly described (Dalmau et al., 2008, Irani et al., 2010b). A high proportion of non-Caucasians were affected which may suggest that genetic factors are involved in the susceptibility to the disease (Irani et al., 2010b, Irani and Vincent, 2011).

The syndrome of anti-NMDAR encephalitis was described to have characteristic temporal progression from the analysis of clinical and paraclinical features of 44 patients (Irani et al., 2010b, Irani and Vincent, 2011). An early “cortical phase” was dominated by psychiatric disturbance, memory and cognitive impairment, and seizures. EEG epileptic changes, MRI cortical changes (rare) and CSF pleocytosis (but not oligoclonal bands) may be seen during this early phase. This is followed after 10-20 days by a “subcortical phase” where movement disorder, autonomic disturbance and reduced level of consciousness are the dominant clinical features. EEG shows diffuse slowing; MRI may show subcortical changes and oligoclonal bands appear in the CSF in the late phase. MRI changes however are absent in about 70% of cases, and abnormalities when present are non-specific consisting mainly of T2 hyperintensities in the white matter (Irani and Vincent, 2011). It was suggested that the early stage represents diffusion of antibodies from serum into the cortical grey matter, and the later stage results from secondary immunological expansion within the intrathecal compartment (Irani et al., 2010b).

Seizures are present in 76 to 83 % of patients with NMDAR- Ab encephalitis and can be focal, focal dyscognitive or generalised (Dalmau et al., 2007, Dalmau et al., 2008, Florance et al., 2009, Irani et al., 2010b). Incomplete syndromes can occur in which one feature predominates including seizures, movement disorder or psychosis (Lancaster et al., 2011b).

A characteristic EEG pattern has been described in some patients with NMDAR encephalitis which consists of generalised slowing in the delta range (1-3 Hz) with superimposed rhythmic fast activity usually in beta range (20-30 Hz). This pattern has been called "extreme delta brush" due to its resemblance to "delta brush" waveforms seen in premature infants (Schmitt et al., 2012), however "extreme delta brush" pattern is more symmetric and synchronous and detected predominantly in frontal regions (Rosenfeld et al., 2012, Armangue et al., 2013).

Immune therapy and resection of the underlying neoplasm when present are proven to provide the best outcome. Relapses can occur in 15-25 % of cases and is thought to be more common in patients without adequate immunotherapy (Gabilondo et al., 2011, Irani and Vincent, 2011).

Anti-NMDA receptor encephalitis has been reported in children and adolescents with a similar phenotype to adult cases (Florance et al., 2009). In children both females and males (to a lesser extent) are affected and tumours are uncommon (Dale et al., 2009b, Florance et al., 2009). The common early symptoms include behavioural and speech problems, seizures, and abnormal movements. Dysautonomia and hypoventilation are less common or severe than in adults (Florance et al., 2009). The syndrome had been recognised for many years in children and given other names such as dyskinetic encephalitis lethargica (Dale et al., 2009b) and immune chorea encephalopathy syndrome (Hartley et al., 2002).

A recent study by Titulaer et al studied a large number of patients (577) including 211 children with NMDAR encephalitis (Titulaer et al., 2013). Median age at disease onset was 21 years (range 8 months to 85 years). 81% of patients were females and male sex was more common in those younger than 12 years and older than 45 years. Similar to previous studies, Titulaer et al showed that tumours were present mostly in females but infrequent in children and men. 220 patients (38%) had an underlying neoplasm (94% of which were ovarian teratomas), of whom 213 (97%) were females, mostly between 12 years and 45 years (46% of all girls and women). Only 6% of girls less than 12 years of age and 6% of men had tumours. The majority of adult patients (65%) presented with behavioural problems while the majority of children younger than 12 years (50%) presented with seizures or movement disorder. Similar spectrum of symptoms was seen within four weeks of onset irrespective of age though central hypoventilation and memory deficits occurred more frequently in adults, while movement disorders and speech problems were common in children. Atypical rare symptoms such as cerebellar ataxia or hemiparesis were more common in children than in adults. Significant neurological improvement was seen in 81% of patients with NMDAR encephalitis in this study by Titulaer et al at 24 months follow up and the factors predicting good outcome were no admission to intensive care unit, early treatment, and low severity of disease. 501 out of the 577 were followed up for longer than 4 months. Twenty nine patients received no treatment with immunotherapy or tumour surgery (due to delayed or retrospective diagnosis). A proportion of these patients showed spontaneous improvement, however 29% had a poor outcome. 472 patients received

first line immunotherapy (steroids, IVIG, plasmapheresis or combination) and tumour removal, when applicable. 251 patients (53% of treated patients) had good outcome. 125 patients out of the remaining 221 patients who did not respond to first line treatment received second-line immunotherapy with rituximab, cyclophosphamide, or both, while 96 had no further immunotherapy or continued to receive first-line immunotherapy. Patients who had second line immunotherapy had better outcome. Relapses occurred in about 12% (which is lower than reported in previous studies) and occurred at higher frequencies in patients who had no tumours and those who received no immunotherapy. The use of immunotherapy particularly second line immunotherapy was associated with reduced relapse rate. Children were similar to the rest of the cohort in prognostic and risk factors (Titulaer et al., 2013).

#### **1.4.4.2 Epilepsy and NMDAR Abs**

In addition to the characteristic NMDAR encephalitis, antibodies to NMDAR were found in women with new onset epilepsy (Niehusmann et al., 2009). Five out of 19 women aged 15-45 years with new onset epilepsy were positive for NMDAR antibodies. Only one patient had ovarian tumour. The five cases positive for NMDAR Abs had extratemporal epilepsies. Associated features included psychiatric disturbance (in four out of five), speech dysfunction and decreased level of consciousness. Features that suggest subcortical CNS involvement were seen in two of the five patients (nyctagmus, dyskinesia, dystonia and hypoventilation). The disease course was episodic and remitting-relapsing. Four of the five positive patients recovered completely either spontaneously or after immunotherapy (Niehusmann et al., 2009).

#### **1.4.5 AMPAR antibodies**

Alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are one member of the glutamate receptors. AMPA receptors mediate most of the fast excitatory neurotransmission in the brain (Shepherd and Huganir, 2007).

Antibodies against the glutamate receptor 1 (GluR1) and GluR2 subunits of AMPA receptor were found in patients with a limbic encephalitis syndrome (Lai et al., 2009). These neuronal surface

antigens were identified using immunohistochemistry and mass spectrometry and were confirmed to be the target antigens using cell based assay. Patients' sera and CSF reacted with human embryonic kidney 293 cells expressing GluR1/2. Application of antibodies to cultures of rat hippocampal neurons caused a small decrease in overall AMPAR cluster density, which was reversed after antibody removal. The ten LE patients positive for AMPAR antibodies were nine women and one man with a median age of 60 years (range 38-87 years). They were selected from 43 patients with LE who had antibodies to unknown cell surface antigens. Four out of the 10 patients with AMPAR Abs had seizures, five had associated systemic autoimmunity and seven had underlying neoplasms. Nine patients received immunotherapy (six of whom also received tumour treatments) and all nine patients responded to treatment given. Five patients had relapses, and one patient died after a prolonged status epilepticus (Lai et al., 2009). Antibodies to AMPAR have not been reported in children.

#### **1.4.6 GABA<sub>B</sub>R antibodies**

Gamma aminobutyric acid-B (GABA<sub>B</sub>) receptors are inhibitory receptors that are involved in regulation of synaptic plasticity. Disruption of GABA<sub>B</sub> receptors has been associated with seizures and memory dysfunction. Antibodies to GABA<sub>B</sub> receptor were found in 15 patients with limbic encephalitis who had seizures as the early or prominent presenting feature (Lancaster et al., 2010). Eight of the 15 patients were men and the median age was 62 years (range 24–75). Seizures were mostly of temporal lobe onset with secondary generalisation, and three patients had status epilepticus. One patient had childhood onset seizures. Seven of the 15 patients had tumours, five of which were small lung cancer (SCLC). Seven patients had other auto antibodies, including to GAD (3 patients), thyroid peroxidase (3 patients), voltage-gated calcium channels (3 patients), and sex determining region Y-box 1 (SOX1) (1 patient). Nine patients had a response to immunotherapy alone and three patients responded to treatment of tumour in addition to immunotherapy (Lancaster et al., 2010).

In a separate cohort, GABA<sub>B</sub>R Abs were also identified in 10 of 70 patients with LE (14%), eight of them had small cell lung carcinoma (SCLC) (Boronat et al., 2011). Nine of the 10 patients were men and the median age was 60 years (range 47–70 years). Seizures were the predominant and presenting

symptom in eight patients and two required intensive care treatment for their seizures. Seven patients were treated with immunotherapy and three of the eight patients with SCLC also received chemotherapy. Only two patients made a complete recovery (Boronat et al., 2011). GABA<sub>B</sub>R Abs have not been reported in children at the time of writing.

#### **1.4.7 GAD antibodies and associated disorders**

GAD (glutamic acid decarboxylase) is an enzyme that catalyses the conversion of L-glutamic acid to gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter (Kwan et al., 2000). GAD is present in GABAergic neurons and in pancreatic  $\beta$ -cells. Antibodies to GAD are present in many disorders including type 1 diabetes mellitus (T1DM) and various neurological disorders particularly stiff person syndrome but also cerebellar ataxia, epilepsy and limbic encephalitis (Solimena et al., 1990, Brown and Marsden, 1999, Peltola et al., 2000, Honnorat et al., 2001, McKnight et al., 2005, Malter et al., 2010). However GAD antibody titres in neurological disorders are much higher than those found in patients with T1DM (Peltola et al., 2000, Saiz et al., 2008). It is thought that GAD antibodies do not have a direct pathogenic role, as GAD is expressed in the cytoplasm rather than external surface of plasma membranes, however they are markers of an immunologic process in these patients with neurologic conditions (Vincent, 2008).

##### ***1.4.7.1 Stiff person syndrome (SPS) and GAD antibodies***

Stiff person syndrome (SPS) is characterised by stiffness and rigidity of axial muscles, exaggerated lumbar lordosis, reflex spasms precipitated by voluntary movements as well as by emotional, tactile and auditory stimuli (Brown and Marsden, 1999). Signs suggestive of brain stem, pyramidal, extrapyramidal or spinal cord pathology are absent in the classic SPS. Patients often respond to diazepam and baclofen and the condition often has good prognosis. The pathophysiology of SPS is unclear though immune mediated mechanisms are thought to play a role. Antibodies to GAD are found in up to 90% of cases of stiff person syndrome, the majority of which are also diabetic (Solimena et al., 1990, Brown and Marsden, 1999). Up to 10 % of SPS patients with GAD Abs also



have epilepsy (Solimena et al., 1990). Paraneoplastic cases of stiff person syndrome have been reported with positive anti amphiphysin antibodies (Pittock et al., 2005).

Stiff person plus syndrome describes severe cases of SPS with encephalomyelitis who also have rigidity that is not confined to the trunk but also involves distal limbs (Brown and Marsden, 1999). Patients have limb myoclonus, brain stem signs, long tract signs, lower motor neuron signs, cognitive and autonomic disturbance and CSF pleocytosis. Post mortem pathology findings showed inflammatory changes in the brain and spinal cord grey matter (Brown and Marsden, 1999). The term progressive encephalomyelitis with rigidity and myoclonus (PERM) is also used to describe these cases (Meinck and Thompson, 2002). Patients with SPS can progress to PERM. PERM can be associated with GAD antibodies less frequently than stiff person syndrome. Paraneoplastic cases of PERM are also described. Some patients with SPS and PERM spectrum were later found to have antibodies to glycine receptors (GlyR), see below (Hutchinson et al., 2008, McKeon et al., 2013). This is an example of a neurologic condition that is known to be associated with GAD antibodies where the GAD antibodies may not be the pathogenic antibodies but may suggest that another pathogenic autoantibody is present such as glycine receptor antibody (a cell surface antigen). Some of the patients with SPS who tested positive to GlyR Abs were also positive for GAD Abs (McKeon et al., 2013).

#### ***1.4.7.2 Limbic encephalitis and GAD antibodies***

Due to the coexistence of type 1 diabetes mellitus in patients with stiff person syndrome and anti GAD antibodies, it was suggested to use a high titre cut-off of GAD antibodies (>2000 unit/ml) in patients with neurological conditions (Saiz et al., 2008). Saiz et al reported high GAD Ab levels in 61 patients, 11 of whom had isolated diabetes and 50 (82%) had neurological conditions including SPS (n=22), cerebellar ataxia (n=17) and other neurological disorders (n=11). The other neurological disorders associated with high titre GAD antibodies in this report included eleven patients with epilepsy, non paraneoplastic limbic encephalitis and paraneoplastic neurological syndromes (Saiz et al., 2008).

Malter et al studied 53 adult patients (17-80 years) with limbic encephalitis (Malter et al., 2010). Nine out of the 53 patients had high-titre GAD Abs. In this report, the nine GAD antibody positive cases were compared to ten VGKC antibody positive cases. Patients with positive GAD Abs in this report were younger than those patients with VGKC Abs, and the illness of the GAD positive patients was dominated by seizures which was often the first sign, rather than cognitive impairment. Interestingly while there was no significant difference in CSF pleocytosis between GAD and VGKC Abs positive cases, the GAD positive cases were more likely to have oligoclonal bands and intrathecal synthesis of GAD antibodies. Mesial temporal MRI abnormalities were seen in both GAD and VGKC antibody positive groups (Malter et al., 2010). The two groups received comparable regimens of immunotherapy (monthly intravenous pulse methylprednisolone), however at 12 months follow up none of the GAD Ab positive group was seizure free, whereas all of the VGKC Ab positive patients were seizure free indicating resistant epilepsy in GAD Ab positive cases. The authors suggested that more intense immunotherapy might be needed in GAD Ab positive LE (Malter et al., 2010).

In children limited reports are available about the GAD Abs in neurological disease. An adolescent 16 year old female with immune limbic encephalitis presenting with new onset temporal lobe seizures and confusion was found to have elevated GAD Abs at >300 unit/ml (Akman et al., 2009). MRI revealed bilateral hippocampal high signal. Her seizures improved with IVIG, steroids and diazepam. The girl was found later to have common variable immune deficiency and steroids were withdrawn which was associated with gradual increase in her seizures. 12 months later her MRI showed mesial temporal sclerosis and she had ongoing seizures (Akman et al., 2009).

#### ***1.4.7.3 Epilepsy and GAD antibodies***

Refractory epilepsy has been reported in association with SPS for many years (Martinelli et al., 1978) and GAD Abs have been found in patients with SPS, diabetes and epilepsy (Solimena et al., 1988). Refractory temporal epilepsy associated with GAD Abs was reported in a 19 year old man who had no diabetes or other organ specific autoimmunity (Giometto et al., 1998). In a separate study, GAD antibodies were found in eight out of 51 patients with focal epilepsy and none of 49 patients with

generalised epilepsy (Peltola et al., 2000). Two of the positive patients had high titre levels of GAD antibodies while six had low levels. The two patients with high GAD Ab levels had therapy resistant temporal lobe epilepsy (Peltola et al., 2000).

In a separate cohort study, low titre GAD Abs were found in six out of 233 patients with epilepsy (Errichiello et al., 2009). Two of the six patients had idiopathic generalised epilepsy with coexisting T1DM and four had cryptogenic temporal lobe epilepsy. Intrathecal synthesis of GAD Abs was not detected in any of these GAD antibody positive patients (Errichiello et al., 2009).

In the McKnight study described above, high levels of GAD antibodies (>1,000 U) were found in 3 out of 139 patients (2.1%) with epilepsy. The GAD Ab positive patients had long-standing drug-resistant epilepsy with young onset (<15 years), focal EEG abnormalities and normal MRIs (McKnight et al., 2005).

#### ***1.4.7.4 Cerebellar Ataxia and GAD antibodies***

High level anti GAD Abs have been previously also reported in patients with cerebellar ataxia (Honnorat et al., 2001). In a lab based study of 9000 serum sample sent from four centres for testing of paraneoplastic neuronal antibodies, 36 samples tested positive for GAD Abs, 22 were from patients with typical stiff person syndrome and 14 were from patients with cerebellar ataxia as the main neurologic feature (Honnorat et al., 2001). The 14 patients with cerebellar ataxia were mostly women (13/14). They all had idiopathic late onset cerebellar ataxia. MRI brain in these GAD positive patients was either normal or showed cerebellar atrophy. All but two of them had other autoimmune disorders including T1DM, thyroiditis and coeliac disease indicating polyendocrine autoimmunity. The authors emphasized the fact that the role of GAD Abs in these patients is unclear and may merely reflect the presence of polyendocrine autoimmunity. However they suggested that the higher GAD Ab titres and the presence of oligoclonal bands and intrathecal synthesis of GAD Abs in these patients may indicate an active immune process in the nervous system (Honnorat et al., 2001).

Cases of cerebellar ataxia associated with epilepsy and GAD antibodies are also reported (Vulliemoz et al., 2007, Nociti et al., 2010). Vulliemoz et al reported a 58 year old man with refractory focal

epilepsy who developed severe ataxia and upbeat nystagmus. In addition to high level GAD Abs the patient had multiple organ specific antibodies including anti-intrinsic factor, anti-thyroglobulin, anti-thyroperoxidase and anti-Langerhans islet cells Abs. The patient's ataxia responded to immunotherapy (steroids and azathioprine) and his seizures responded to the addition of benzodiazepines (Vulliemoz et al., 2007). A similar case of cerebellar ataxia and epilepsy with GAD antibodies was reported by Nociti et al in 2010. This was a 42 year old woman with type-1 diabetes mellitus, Hashimoto's thyroiditis, vitamin B12 deficiency, horizontal and upbeat nystagmus, cerebellar ataxia and drug resistant generalised seizures associated with anti-GAD Abs. Neurologic symptoms and seizures did not improve with B12 replacement and multiple anti-epileptic drugs, however they did improve with immunotherapy in the form of steroids and azathioprine (Nociti et al., 2010).

#### **1.4.8 Glycine receptor antibodies**

Antibodies to glycine receptor (GlyR) have been described in patients with progressive encephalomyelitis with rigidity and myoclonus (PERM) (Hutchinson et al., 2008, Mas et al., 2011, Leite et al., 2012), as well as in patients with classic stiff person syndrome or SPS plus (McKeon et al., 2013). It is interesting to note that mutations in the  $\alpha 1$  subunit of the glycine receptor gene *GLRA1* have been identified in hereditary hyperekplexia, which is characterised by stimulus sensitive myoclonus (Shiang et al., 1993).

Myoclonus in PERM is thought to be non-epileptic in origin. Seizures however have been reported in association with GlyR antibodies. A rapidly fatal case with PERM was reported in a 28 year old man who had antibodies against both GlyR and NMDAR (Turner et al., 2011). Prior to progression into rigidity, myoclonus and hyperekplexia he presented with suspected generalised seizures and his EEG showed frequent left temporal sharp waves. Post mortem examination revealed encephalomyelitis, with particular involvement of hippocampal, pyramidal and cerebellar Purkinje cells and relative sparing of the neocortex (Turner et al., 2011).

A case of immunotherapy-responsive LE associated with GlyR Abs presenting with refractory convulsive status epilepticus was reported in a 25 y old Indian man (Zuliani et al., 2011). The authors suggested that GlyR Abs can affect limbic areas, as glycine receptors which are prominent in the brainstem and spinal cord, are also expressed in the hippocampus (Zuliani et al., 2011).

In children one case of PERM associated with glycine receptor antibodies has been reported in a 14 month old female infant who developed startle-induced episodes of generalised rigidity and myoclonus and axial hyperextension without impairment of consciousness (Damasio et al., 2013). Investigations were negative but glycine receptor antibodies were detected in serum and CSF. This case responded to immunotherapy in the form of IVIG and steroids; however she had relapses of symptoms.

#### **1.4.9 Summary of neuronal antibodies associated with seizures**

Antibodies to specific neuronal cell surface membrane proteins and GAD are found in many CNS disorders where seizures are important feature including different forms of encephalitis as well as epilepsy. These antibodies and associated disorders in children and adults are presented in Table 1-2 (Graus et al., 2010, Vincent et al., 2010, Lancaster and Dalmau, 2012). This area is evolving and new reports are added regularly.

*Table 1-2 Neuronal antibodies and associated phenotypes in adults and children*

| Antibody type       | Associated disorders in adults                                  | Associations with tumours (mostly reported in adults) | Associated disorders in children  |
|---------------------|---|---|---|
| VGKC                | Limbic encephalitis, seizures, Morvan's syndrome, neuromyotonia | Rare (thymoma, SCLC)                                  | Limbic encephalitis, seizures, status epilepticus, development regression |
| LGI1                | LE, FBDS  | Rare (lung cancer, thymoma)                           | Not reported  |
| CASPR2              | Morvan Syndrome, neuromyotonia, LE                              | Thymoma, lung cancer                                  | Not reported  |
| NMDAR               | Encephalitis, Epilepsy, psychiatric disturbance                 | Ovarian teratoma                                      | Encephalitis, movement disorder, psychiatric disturbance                  |
| GAD                 | LE, epilepsy, stiff person syndrome                             | Rare (lung cancer)                                    | LE  |
| GABA <sub>B</sub> R | LE, seizures, memory loss                                       | SCLC, thymus  | Not reported  |
| AMPA                | LE, psychiatric disorders                                       | Lung, breast, thymus                                  | Not reported  |
| GlyR                | Stiff person syndrome, PERM, myoclonic seizures, LE             | Rare (lung cancer, thymoma)                           | Rare (one case of PERM)   |

LE: limbic encephalitis

FBDS: faciobrachial dystonic seizures

PERM: progressive encephalomyelitis, rigidity and myoclonus

SCLC: small cell lung cancer

#### **1.4.10 Methods in use for detection and measurement of neuronal antibodies**

VGKC antibodies are measured by radioimmunoassay (RIA) using mammalian brain tissue derived VGKCs labelled with iodinated dendrotoxin, a snake toxin specific for some subtypes of the Kv1 family of potassium channels (subunits Kv1.1, 1.2 and 1.6) (Hart et al., 1997). The dendrotoxin-labelled VGKCs immunoprecipitated by patient antibodies were later found to be a complex of at least three accessory surface proteins, LGI1, CASPR2 and contactin 2, that are closely complexed with the VGKC, hence the name 'VGKC-complex' (Irani et al., 2010a, Lai et al., 2010, Irani et al., 2011a). Antibodies against these proteins within the VGKC complex (LGI-1, CASPR2 and contactin-2) are detected using immunofluorescence cell based assays (CBA) which detects the binding of

patients' sera to the surface of cells transfected with cDNA encoding the relevant protein. This method, cell based assays using transfected cells is now the main method used for testing for antibodies against: LGI 1, CASPR2, NMDAR, GABAR, AMPAR and Gly-R. A microscopy scoring system is used and the binding is scored visually from 0 (no binding) to 4 (very strong binding) by two independent observers (Irani et al., 2010b).

As for NMDAR, cell based assays done at different institutions have used different dilutions of serum and CSF. The Oxford Group uses a higher concentration of serum with a dilution ratio of 1:20 and undiluted CSF, as compared to Dalmau group who uses serum at 1:200 dilutions and CSF at 1:10 dilution (Dalmau et al., 2008, Irani and Vincent, 2011).

RIA, which is still used to measure VGKC Abs, is also used to measure antibodies against GAD, using <sup>125</sup>I-labelled human recombinant GAD 65 (Saiz et al., 2008).

Immunohistochemistry (IHC) maybe used first as a screening method to identify antibody binding in patients sera to brain tissue. This is followed by a quantitative assay specific to different antibodies as described above (CBA or RIA).

The different methods that are currently in use for the detection of antibodies against different neuronal antigens are presented in Table 1-3

*Table 1-3 Methods in use for neuronal antibodies detection and assays*

*(adapted from Vincent et al 2011).*

|              | IHC | RIA | CBA | ELISA |
|--------------|-----|-----|-----|-------|
| VGKC complex | +   | +   |     |       |
| LGI1         | +   |     | +   |       |
| CASPR2       | +   |     | +   |       |
| NMDAR        | +   |     | +   | +     |
| GAD          | +   | +   |     | +     |
| GlyR         |     |     | +   |       |

IHC: immunohistochemistry

RIA: radio-immuno assay

CBA: cell based assay

ELISA: enzyme linked immunosorbent assay



## 1.5 Principles of pathogenic antibodies

The knowledge about the role of antibodies in diseases of the central nervous system is expanding.

While the presence of autoantibodies in the CNS is usually associated with disease “disease associated antibodies”, there are also the so called “naturally occurring antibodies” which can be important in brain homeostasis, such as anti  $\beta$ -Amyloid antibodies which clear  $\beta$ -Amyloid aggregates and prevent their neurotoxicity (Gold et al., 2012).

The presence “disease associated antibodies” alone does not necessarily establish a causal link between the antibodies and the disease (Archelos and Hartung, 2000). To prove the pathogenic role of autoantibodies in the disorders of the peripheral and central nervous systems many criteria need to be fulfilled which include “in addition to the presence of the antibodies”: demonstration of antibodies binding to a target antigen at the site of pathology and clinical benefit following removal of antibodies by plasma exchange. In addition the passive transfer of antibodies in question to experimental animals should transfer the disease phenotype (Archelos and Hartung, 2000).

It is thought that antibodies against surface antigens including VGKC, LGI, CASPR2, NMDAR, AMPAR, GABA<sub>B</sub>R and GlyR are likely to be pathogenic and usually predict immunotherapy responsive and reversible syndromes that are not frequently associated with neoplasms. In contrast antibodies against intracellular antigens (cytoplasmic or nuclear) such as Hu, Yo, CV2, Ma2 etc which are found in paraneoplastic disorders such as limbic encephalitis are thought to be markers of the associated tumours, rather than directly pathogenic autoantibodies involved in the CNS syndrome. These antibodies are called onconeural Abs due to their frequent association with tumours. It is thought that cytotoxic T cell mediated immunity is involved in paraneoplastic neurological syndromes rather than an antibody mediated process. Onconeural antibodies, which are more relevant to adults than paediatric population, are typically associated with treatment unresponsiveness and poor outcome (Irani and Vincent, 2011).

GAD antibodies are targeted against an intracellular antigen, and it is unclear whether GAD antibodies are pathogenic or not. It has been suggested that GAD could be exposed on the cell surface

during exocytosis from GABA-ergic neurons, allowing a pathogenic antibody-antigen interaction (Malter et al., 2010). Other theories to explain the underlying immune mechanisms associated with GAD Abs include T-cell-mediated mechanisms (Lancaster and Dalmau, 2012). The presence of other antibodies in the sera of patients positive for GAD antibodies that bind to unknown surface antigens has also been suggested (Vincent et al., 2011b). Nevertheless, GAD antibodies are thought to be useful markers for immune mediated forms of epilepsy or limbic encephalitis and some of these syndromes are responsive to immunotherapy (Vincent et al., 2010).

Intrathecal synthesis of antibodies may be a useful marker to the pathogenicity of antibodies detected in serum. It is proposed that CSF analysis improves the sensitivity and specificity of testing, particularly in NMDAR encephalitis (Lancaster and Dalmau, 2012). NMDAR Abs are often detected in CSF as well as serum (Dalmau et al., 2007, Dalmau et al., 2011). GAD antibodies were frequently detected in CSF as well as in serum of patients with variable neurologic conditions including LE and epilepsy (Saiz et al., 2008). The significance of this finding is debatable, and intrathecal synthesis of antibodies is not found in other syndromes associated with other autoantibodies such as VGKC and LGI1 antibodies (Vincent, 2008). The lack of intrathecal synthesis, as is the case in VGKC complex antibody associated limbic encephalitis, does not exclude an immune mediated process (Jarius et al., 2008, Vincent, 2008).

## **1.6 Epilepsy and other autoimmune diseases**

Epilepsy is reported in association with many systemic autoimmune disorders including multiple sclerosis, systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), coeliac disease and autoimmune thyroid disease (Palace and Lang, 2000, Vincent and Crino, 2011). In most cases the mechanisms of this association between autoimmune disorders and epilepsy is not clearly understood. It is possible that pathogenic neuronal antibodies might be present and responsible for the CNS involvement. However other causes such as coincidental coexistence, genetic predisposition for both conditions, or secondary effects of the primary disease might be the cause of epilepsy in these conditions (Vincent and Crino, 2011).

## 1.7 Treatment of autoimmune seizures and encephalopathies

Many studies have shown that seizures of autoimmune origin respond to immune therapy better than to conventional antiepileptic drugs.

There are currently no standard immunotherapy protocols for the treatment of immune mediated seizures, although similar regimens are used worldwide (Wong et al., 2010, Wong-Kisiel et al., 2012). Most people agree to the use of first line immunotherapy, which consist of steroids (usually in the form of high dose intravenous methyl prednisolone), intravenous immunoglobulin and plasmapheresis. These agents are used individually or in combination. Plasmapheresis use in children is limited due to its invasiveness and is usually limited to cases that do not respond to steroids, or to steroids and IVIG. This regimen is usually followed by variable length duration of tapered oral steroids. There is also consensus that failure of first line immunotherapy is an indication for consideration of second line immunotherapy, which consists of one or more of the immunosuppressant agents such as cyclophosphamide, mycophenolate or rituximab. Second line immunotherapy decision-making is often influenced by the treating clinicians' previous experience, and cost versus benefit analyses.

In addition to acute term treatment and treatment escalation to second line immunotherapy in those who do not respond to first line immunotherapy, long term treatment is also adopted by some clinicians although there are no guidelines available. For example long term treatment with immunosuppression, such as mycophenolate or azathioprine for 12 months or so might be used for patients who responded to these treatment in order to prevent relapse. There is currently no evidence to support this practice.

In addition to immune therapy, treatment of any underlying tumour is important to achieve good outcome (Dalmau et al., 2011).

It is well recognised that early and aggressive treatment of these disorders, including the use of second line treatment when the first line fails, will provide the best outcome. A recent large review of NMDAR encephalitis supports this approach (Titulaer et al., 2013).

## **1.8 Vulnerability to autoimmune seizures and encephalopathies**

Many factors may give an individual a higher susceptibility to develop one or more of the autoimmune seizures and encephalopathies. The predisposing factors for many of the Ab autoimmune seizure disorders include genetic and environmental factors, and it is likely that this vulnerability and interaction between these factors is complex, as is the case in autoimmune diseases in general. The following factors are likely to be important:

1. Ethnic origin, as NMDAR encephalitis is more common in non-Caucasian individuals, which may reflect an underlying genetic susceptibility (Irani and Vincent, 2011).
2. Female sex, which is true of most autoimmune diseases and for most disorders associated with neuronal Abs except for VGKC encephalitis which is more common in males (Vincent et al., 2004, Vincent et al., 2010).
3. The association with neoplasm, as is the case in NMDAR encephalitis, AMPAR Ab associated encephalitis and in some cases of VGKC-complex Ab encephalitis (particularly in association with CASPR2). Antigenic targets are expressed by the tumours, which provide the antigenic stimulus to produce the relevant antibodies and result in the neurological syndrome. The neurological syndrome might precede the diagnosis of the tumour, and occult tumours might be present in apparently 'non-paraneoplastic' cases (Irani et al., 2010b). The association with tumours is less common in children than in adults.

Black women with NMDAR encephalitis are more likely to have ovarian teratomas (Dalmau et al., 2011, Titulaer et al., 2013). This might reflect that genetic susceptibility might also play a role in the predisposition to autoimmune response in the presence of neoplasms.

4. Preceding or concomitant infections which may act as a trigger for the autoimmune response.

Serological evidence of mycoplasma infection is not uncommon in association with NMDAR encephalitis in children, though the significance of this finding is unclear (Florance et al., 2009, Gable et al., 2009).

5. The association with other autoimmune diseases or autoimmune markers, which may indicate an increased genetic susceptibility to immune activation. Antinuclear antibodies are present in patients with VGKC encephalitis (Vincent et al., 2004, Suleiman et al., 2011a) and elevated ANA and thyroid Abs were found in some children with NMDAR encephalitis (Florance et al., 2009). T1DM and other autoimmune endocrinopathies are often present in association with neurological conditions where GAD Abs are thought to play a role.

## **1.9 Study hypothesis and aims**

The findings of neuronal Abs in adult patients with seizures and epilepsy including limbic encephalitis, NMDAR encephalitis and autoimmune epilepsy prompted us to study these antibodies in children with seizures and epilepsies. The disorders associated with neuronal Abs are potentially treatable and reversible with early and prompt immune therapy. Therefore it is important to study their role in children to improve recognition, treatment and outcome of these disorders.

This thesis examines the following hypotheses:

1. A proportion of paediatric epilepsies are autoimmune in origin.
2. Neuronal Abs play an important role in a proportion of children with seizures and epilepsy.
3. Neuronal autoantibodies are associated with particular epilepsy phenotypes.
4. These autoimmune childhood epilepsies might be treatable and reversible with immunotherapy, if recognised and treated promptly.

The aims of this study are as follows:

1. To study the presence of neuronal Abs in childhood epilepsy and seizure disorders.

2. To recruit a large cohort of patients with epilepsy, study their demographics and clinical features and classify them using standard ILAE system.
3. To test the sera of these patients for autoantibodies against neuronal antigens and proteins including VGKC complex and its various components (LGI1, CASPR2, contactin2), NMDAR, GlyR and GAD (in addition to AMPAR, GABA<sub>B</sub>R which became available later in the course of this study).
4. To define the epilepsy phenotypes associated with the different neuronal Abs in children
5. To study the patients' response to immune therapy (when given) and their outcome.

# Chapter 2 VGKC-complex Antibody Associated Encephalitis in Children

## Acknowledgment

**This chapter is a detailed and a slightly modified version of the published work in paper 1 in Appendix 6 (Suleiman et al., 2011a).**

## 2.1 Introduction

At the time of conducting this study in 2009, VGKC Ab associated encephalitis was not yet reported in children. In order to present the background to the current study, we present the background of VGKC-associated encephalitis in adults. Vincent et al 2004 described 10 patients predominantly males, above 40 years, with VGKC Ab associated LE (Vincent et al., 2004). These patients presented with subacute memory disturbance and confusion as well as seizures. Seizures were focal or generalised and were severe enough to require intensive care treatment in some patients. Most patients had significant memory disturbance on formal testing. MRI showed hippocampal and temporal lobe abnormalities in eight out of the 10 patients described by Vincent et al. Hyponatraemia was found in most of the patients described by Vincent et al and was attributed to syndrome of inappropriate antidiuretic hormone secretion (SIADH), which was sometimes resistant to treatment. Cerebrospinal fluid (CSF) was normal or only mildly abnormal. CSF pleocytosis was present in five of the ten cases and CSF oligoclonal bands were present in five out of the eight patients tested, with matching serum bands in four. EEGs showed non-specific changes including slowing, which was mostly generalised (but focal temporal in one case) and focal sharp waves mainly in the temporal regions in some cases. Neuromyotonia was found on electromyography (EMG) in one patient out of eight tested.

As limbic encephalitis was known to be mostly a paraneoplastic syndrome, the sera of the 10 patients described by Vincent in 2004 were tested for the known onconeuronal antibodies and were negative. However some sera (two cases) showed a characteristic pattern of binding to the rat cerebellum on immunohistochemistry with strong binding to the molecular layer of the rat cerebellum and sparing of

the Purkinje cells. This pattern was different to any of the binding patterns of the known paraneoplastic antibodies but similar to a pattern found previously in some patients with Morvan's syndrome who had high VGKC-Abs. VGKC-Ab titres in all 10 patients were measured by the method in place at the time radioimmunoassay using whole rabbit-brain homogenate. The VGKC-Ab titre in the 10 patients ranged from 450 to 5128 pM (neurological and healthy controls <100 pM). Those with very high level (>2000pm) showed the same characteristic binding pattern described above on immunohistochemistry (positive). There was no evidence of a para-neoplastic process in any of the patients. The 10 patients described by Vincent et al 2004 received immune therapy using variable regimens including steroids, intravenous immunoglobulins and plasma exchange at variable times during their illnesses. These treatments were associated with falls in serum level of VGKC Ab, which also correlated with improvement in the neuropsychological profile in most patients. Of note was the rapid fall in serum Ab levels in patients who received steroid treatment early in the disease course, compared to a much slower fall in those patients who received steroids late or did not receive any. Nevertheless variable degrees of brain atrophy particularly involving the medial temporal structures as well as residual cognitive impairment were seen in some patients. This form of LE was thought to be immune responsive with good prognosis compared to the paraneoplastic LE. At present it is well recognised that early and aggressive treatment provides the best chance for a better outcome in VGKC Ab associated encephalitis (Wong et al., 2010).

Subsequent studies showed that the VGKC antibodies found in patients with LE were in fact mostly targeted at other proteins that are tightly associated with the potassium channel rather than the channel itself, namely LGI1, CASPR2 and contctin2. The term VGKC-complex is now used to describe the VGKC and its associated proteins (see Chapter 1) (Irani et al., 2010a, Lai et al., 2010).

Our index case and motivation for this study was a child at the Children's Hospital at Westmead (CHW) who presented with encephalopathy along with status epilepticus and refractory seizures in November 2008. She was thought to have encephalitis however no infectious cause was found. Her case history is presented in details below. We thought that her presentation was reminiscent of the adult patients described with VGKC associated limbic encephalitis. Given the refractory nature of her



illness, she was tested for VGKC Abs and was found to be positive. She was the first child we identified with VGKC encephalitis. Her case prompted us to look for more cases in children presenting with unexplained encephalitis and status epilepticus. Although at the time only described in adults, we hypothesised that VGKC Abs associated encephalitis could potentially affect children as well. Based on the adult findings, we hypothesised that it was likely that affected children were at risk of poor outcome if not identified and treated early with appropriate immune therapy.

## **2.2 Case Report of index case**

We report a paediatric case of VGKC-complex antibody associated encephalitis, presenting with status epilepticus, encephalopathy, dysautonomia and cognitive impairment. I have published this case in brief as part of the work arising from this PhD (Suleiman et al., 2011a), (see Appendix 6).

### **2.2.1 Presentation of index case**

A 14 year old girl of Vietnamese origin presented with a subacute illness progressing over 14 days of headache, dizziness and confusion followed by new onset focal motor seizures with secondary generalised tonic-clonic seizures (GTCS) (Table 2-1). On presentation to the local hospital she was suspected to have encephalitis and was treated with cefotaxime and acyclovir. The seizures did not respond to intravenous boluses of midazolam, phenytoin and phenobarbitone. She was transferred to our tertiary children's hospital. On arrival she was encephalopathic and combative. She had a low grade fever but no focal neurologic deficit. She continued to have frequent GTCS lasting a few minutes every 30-90 minutes without intervening return of consciousness. She was intubated, ventilated and commenced on a midazolam infusion. There was no family history of epilepsy or febrile seizures.

### **2.2.2 Investigations of index case**

The initial EEG showed left periodic epileptic discharges whilst subsequent EEGs showed generalised slowing and alternating right and left centro-temporal electrical seizures (Figure 2.1).

Video EEG monitoring performed during the first 5 days of hospital admission revealed diffuse slowing at 0.5-2 Hz with periodic focal sharp fast activity sometimes preceding the onset of focal electric seizures. Many focal seizures were captured with electrical onset from right or left centro-temporal regions (independently). Clinically the seizures consisted of eye blinking, eye deviation, eye rolling, peri-oral automatism and clonic upper limb jerking.

Brain MRI at presentation and three weeks into her admission was normal. Initial CSF examination revealed two polymorphonuclear and four mononuclear cells per mm<sup>3</sup>, normal protein and glucose, and negative PCR for herpes simplex virus and enterovirus. CSF neopterin was elevated at 87.9nmol/L (normal 7-28) supporting the suspicion of an inflammatory process. Oligoclonal bands were not detected in CSF. The erythrocyte sedimentation rate was 40 mm/hr (normal <20), and C-reactive protein was 42 mg/L (normal 0-10). Hyponatraemia was not a feature of the biochemistry. Liver transaminases were mildly elevated transiently. Serology for mycoplasma, cytomegalovirus, Epstein Barr virus, human herpes virus 6, toxoplasma, and cat scratch disease were negative. Anti-nuclear antibody (ANA) titre was elevated at 1:640 with a speckled pattern. However, further autoimmune testing including complement, anticardiolipin and antibodies against extranuclear antigen, double stranded DNA, thyroglobulin and thyroid peroxidase antibodies were negative. Pelvic ultrasound revealed no ovarian teratoma.



**Figure 2.1** EEG of index case showing generalised background slowing

(A) A focal electrical seizure of 90 second duration arises independently from the right centrotemporal region. (B) Two minutes later, a focal electrical seizure arises from the left temporal region. The electrical seizures were clinically associated with focal motor seizures characterised by blinking, eye rolling, oral automatisms, facial and limb jerking. “The recording is from day 4 of hospitalisation”.

*Table 2-1 Summary of the clinical features and investigations of index case*

|  |   |
|--|---|
| <p><b>Clinical features of index case</b></p>  | <p><b>Headache</b></p> <p><b>Dizziness</b></p> <p><b>Seizures and status epilepticus</b></p> <p style="padding-left: 40px;"><b>Focal (temporal lobe onset)</b></p> <p style="padding-left: 40px;"><b>Generalised tonic clonic (secondary)</b></p> <p><b>Autonomic instability (hyperthermia and hypotension)</b></p> <p><b>Confusion, agitation, behavioural alteration and emotional lability</b></p> <p><b>Cognitive impairment</b></p> |
| <p><b>Investigations of index case</b></p> <p style="padding-left: 40px;"><b>EEG</b></p> <p style="padding-left: 40px;"><b>MRI</b></p> <p style="padding-left: 40px;"><b>CSF</b></p> | <p><b>Encephalopathic, generalised slowing</b></p> <p><b>Focal periodic epileptic discharges</b></p> <p><b>Alternating centro- temporal electrical seizures</b></p> <p><b>Normal</b></p> <p><b>Mild pleocytosis (6 WBC cells per mm<sup>3</sup>)</b></p> <p><b>High neopterin (87.9nmol/L)</b></p>  |

### **2.2.3 Clinical Progress of index case**

During her 18 days of intensive care stay she had autonomic instability with hyperthermia up to 41.5°C requiring vecuronium and cooling blankets, and hypotension requiring inotropic treatment. Extensive work up to look for a source of sepsis as a cause of high fever and hypotension was negative. Despite midazolam infusion, sodium valproate, phenytoin and levetiracetam, she continued to have up to 20 complex partial and generalised seizures per day. She had transient elevation of

hepatic transaminases which improved on withdrawal of phenytoin. She also had a generalised erythematous rash which was thought to be viral or drug related.

Her seizures became less frequent from day 13 of hospital admission onwards and she was extubated and discharged to the ward at day 18. She was emotionally labile and had word finding difficulties. She was discharged home after a 25 day admission on levetiracetam and clobazam.

The diagnosis of VGKC encephalitis was made retrospectively six months after the acute illness. Her serum VGKC antibodies from the acute illness (collected during intensive care stay) were 847 pM (normal <100), and remained elevated on convalescent testing after six months (679 pM). Antibodies against LGI-1, CASPR2, NMDA receptor and GAD were negative.

#### **2.2.4 Outcome of index case**

Formal neuropsychology assessment at three months revealed impairments of cognition and higher executive function, including problems with emotional control, attention and complex thinking. At six months, she had no further seizures and anti-epileptic drugs (levetiracetam and clobazam) were weaned. However she continued to have significant word finding difficulties, memory impairment and her school performance has deteriorated. A repeat ANA was down to 1:160.

In view of the ongoing cognitive concerns and elevated VGKC Ab 14 months after her acute illness, she was given one course of intravenous immunoglobulin and oral steroids for 1 month. At 15 months follow-up after her encephalitis illness, she was back to her pre-morbid functioning according to her family and school, and has been weaned off all anti-epileptic drugs, and no further immunotherapy was given. Formal neuropsychology testing was not performed at the time.

At the time of writing- 3 years following the acute illness, further follow up by her adult neurologist reported that she re-developed seizures. Her VGKC antibodies retested were low positive (150 pM), and she is receiving further monthly intravenous immunoglobulin.

### 2.2.5 Discussion of index case

The clinical syndrome of our paediatric patient was similar to VGKC encephalopathy described in adults (Thieben et al., 2004, Vincent et al., 2004). Adults with VGKC encephalopathy may have associated autoimmune disorders (Tan et al., 2008), and our patient had transient elevation of ANA acutely but the remaining auto-antibody testing was negative. The CSF in our patient was abnormal with mild pleocytosis and elevated neopterin. It is recognised that the CSF is often normal or only mildly abnormal in VGKC encephalitis. In adults MRI may reveal hippocampal and temporal lobe abnormalities and some patients have hyponatraemia, although both were absent in our paediatric patient.

The clinical course of our patient was complicated by refractory status epilepticus and dysautonomia. We initially feared she had Devastating Epileptic Encephalopathy in School-Aged Children (DESC) or Febrile infection-related epilepsy syndrome (FIRES) (Mikaeloff et al., 2006, van Baalen et al., 2009). DESC/FIRES is a pseudo-encephalitis syndrome of unknown cause that affects previously well children and has severe morbidity and mortality. The subsequent course in our patient was however not consistent with DESC or FIRES. Although our patient made significant spontaneous improvement in her seizure control, she had residual cognitive impairment. In view of the continuing positive VGKC antibodies and her cognitive impairment, she was given intravenous immunoglobulin therapy 2 g/kg over 2 days (one course only) followed by oral steroids 60mg/day for 2 days then a one month tapering course, This regimen which is a relatively short course of immunotherapy was used as she was thought to have returned to her pre-morbid state at the time of treatment 15 months following the acute illness. One could argue that this treatment was probably inadequate particularly given the fact that her seizures have recurred 20 months later.

In conclusion, this was the first reported description of encephalitis with high titre VGKC antibodies in a child. We can conclude that VGKC Ab associated encephalitis occurs in children, and should be considered a cause of new onset severe epilepsy and status epilepticus.

## **2.3 Hypothesis and aim**

The increasingly recognised syndrome of VGKC Ab associated LE in adults and the positive result in our index case prompted us to look for further cases in children. We wondered if VGKC antibodies have an important unrecognised role in some paediatric patients who present with unexplained encephalopathy or encephalitis as well as seizures and status epilepticus. We wished to determine if this treatable form of encephalitis affects children.

We aimed to study a cohort of paediatric patients with unexplained encephalitis and new onset status epilepticus, and test them for VGKC antibodies as well as other neuronal antibodies including N-methyl-D-aspartate receptor (NMDAR), glutamic acid decarboxylase (GAD), and glycine receptor (GlyR) antibodies. AMPAR and GABAR antibody testing was not available in 2009/2010 at the time of this study.

## **2.4 Methods**

### **2.4.1 Patients collection**

Based on the findings in our index case who presented with encephalitis and status epilepticus, we decided to look retrospectively for further similar cases. Initially we looked for patients who presented with status epilepticus to the CHW between January 2003 and June 2009 by searching our hospital medical records. We used the following International Classifications of Diseases (ICD)-10 codes: G41.0, G41.1, G41.2, G41.8 and G41.9 (WHO, 2010). 141 patients were identified with status epilepticus; only 36 had serum available for testing. We also looked for patients with encephalitis during the same time period using the following ICD-10 codes: G04.0, G04.8 and G04.9 and we selected those who had severe seizures or status epilepticus on presentation. Four more patients were identified with encephalitis and status epilepticus or severe seizures. The clinical data for the 40 patients were studied and 13 patients with both encephalitis and status epilepticus (as defined below) who had serum available for testing were identified. Three of the 13 patients with encephalitis and status epilepticus had a defined aetiology for their encephalitis (Enterovirus n=2, H1N1 influenza

n=1) and were excluded leaving 10 patients with unexplained encephalitis and status epilepticus who were included in this study.

We used the clinical criteria for encephalitis as defined by Glaser et al. (Glaser et al., 2003). This definition has been accepted and is used in large contemporary encephalitis cohorts (Granerod et al., 2010). A case of encephalitis was defined by the presence of encephalopathy (depressed or altered level of consciousness lasting more than 24 hours, lethargy, or change in personality or behaviour) with 2 or more of the following symptoms: fever, seizure, focal neurologic findings, CSF pleocytosis, or electroencephalograph or neuroimaging findings consistent with encephalitis (Glaser et al., 2003).

Status epilepticus (SE) was defined as a single seizure longer than 30 min or series of seizures without recovery of function in between lasting more than 30 min (Commission On et al., 1993).

Approval by the Ethics Committee at The Children Hospital at Westmead was obtained as part of the approval for the autoimmune epilepsy study in children (see Appendix 3). Written consents to test acute stored sera were obtained from the patients or their families.

Patients' data were studied in detail using the electronic medical records system, including demographics (sex, age), past medical history and co-morbidities, presentation clinical information including seizures semiology/phenomenology, status duration, associated illness features, admission to hospital and intensive care unit, investigations performed (EEG, imaging, CSF, serology) and treatment given (see Appendix 4). The following associated illness features were recorded if reported by the carers or clinicians:

- Encephalopathy, which was defined by the presence of lethargy, drowsiness or altered responsiveness.
- Behavioural alteration, defined by the presence of agitation, emotional lability or irritability.
- Cognitive alteration which was difficult to ascertain particularly for young children and infants and was defined by the presence of memory or intellectual concerns.



Given the retrospective nature of the study some clinical data were difficult to obtain with certainty by reviewing the clinical records including seizure semiology, status duration, as well as the presence of associated illness. Where no clinical information was recorded these features were considered absent.

Clinical data were further studied and seizures were classified according to ILAE classification (Berg 2010). Seizure types were categorised into focal onset, focal with secondary generalisation or generalised at onset.

Sera stored from the acute illness at the hospital laboratory were retrieved and stored for shipment to our collaborators for antibody assays. This was a collaborative study with Angela Vincent and Bethan Lang, Oxford UK who were one of the few labs in the world to perform VGKC-complex and other neuronal antibodies testing (methods below).

#### **2.4.2 Clinical features of cohort (n=10)**

The 10 patients with unexplained encephalitis and refractory seizures/status epilepticus (4 males, mean age 7.5 years, range 1–14) had no previous seizures; eight had no preceding neurologic abnormality and two had preceding mild developmental delay and learning difficulty. The mean length of hospital stay for the encephalitis event was 19.7 days (median 24.5 days, range 6–36). Nine of the 10 patients (90%) were admitted to the intensive care unit (ICU); the mean length of ICU stay was 9 days (median 5 days, range 2–25). The SE was convulsive in all 10 patients, focal in 2, generalised in 6, and secondary generalised in 2. All patients had encephalopathy, eight had behavioural alteration and five had cognitive alteration. Eight patients had fever, and one had diarrhoea.

#### **2.4.3 Investigations of cohort (n=10)**

All 10 patients had EEG which was abnormally slow in all with focal features in seven, epileptic activity was seen in four and electrical seizures in one (see Tables 2-3 & 2-4). All 10 patients had CSF examination including microscopy, culture, and glucose and protein measurement. CSF pleocytosis (defined as more than 5 white cells per mm<sup>3</sup>) was present in nine out of the 10 patients. The mean

CSF white cell count was 14.7 cells per mm<sup>3</sup>). CSF protein was elevated (> 0.40 g/dl) in 5 patients. The mean CSF protein was 0.38 g/dl. CSF polymerase chain reaction (PCR) for Herpes Simplex Virus (HSV) and enterovirus was negative in eight patients tested. Serological testing including: mycoplasma pneumoniae (n=9), enterovirus (n=7), cytomegalovirus (n=6), Epstein-Barr virus (n=6), herpes simplex virus (n=5), human herpes virus 6 (n=3), influenza (n=4), and adenovirus (n=3) were negative.

Magnetic Resonance Imaging (MRI) of the brain was performed acutely in all 10 patients, three were normal. The abnormal features on MRI consisted of the following: leptomeningeal enhancement (n=4), cerebral oedema (n=3) and white matter signal abnormality (n=2). Follow up MRI scans showed brain atrophy in one patient and mesial-temporal sclerosis in another.

#### **2.4.4 Control group**

The reference ranges for VGKC Ab testing were described only in adults and there was no reference range for children. We are aware that children's normal titres might be different to adults hence we aimed to define a paediatric control reference range.

We used 69 childhood controls including 15 healthy children (8 male, mean age 11 years, range 9–13 years), 14 with non-inflammatory neurologic disorders (6 male, mean age 7.8 years, range 3–15 years), 19 with immune mediated ataxia (11 male, mean age 6 years, range 1–11 years), 10 with encephalitis lethargica and parkinsonian features (6 male, mean age 9.4 years, range 5–15 years), and 11 with NMDAR encephalitis (2 male, mean age 6.53 years, range 1.3–13 years). These patients were collected over the last 10 years as part of testing to define specificity of novel auto-antibodies.

#### **2.4.5 VGKC- complex antibody and other neuronal antibodies assays**

The stored serum used for neuronal antibody testing was acute serum from the first week of the encephalitis admission in all 10 patients. Sera were tested in Oxford by our collaborators for specific neuronal antibodies known at the time of the study including the following: VGKC complex, NMDAR, glutamic acid decarboxylase (GAD), and glycine receptor (GlyR) antibodies. Sera were

also tested for Ab against leucine-rich glioma-inactivated 1(LGI1) and contactin-associated protein-like 2 (CASPR2); which were identified to be tightly complexed with VGKCs in vivo (Irani et al., 2010a, Lai et al., 2010).

#### **2.4.5.1 Methods used for antibodies assays**

The following section (in italic) was written by Dr Sukhvir Wright, Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, United Kingdom, and describes the methods used for neuronal antibodies assays at their laboratory. These methods were used for antibody assays in the different studies done as part of this PhD project (this Chapter, Chapter 3 and 4).

##### **2.4.5.1.1 Cell-based assay (CBA)**

*This method was used for detection of cell surface antibodies to the NMDA, glycine receptors, and Lgi1 and Caspr2 proteins (as well as contactin2 and AMPAR in the later studies).*

*Human embryonic kidney cells (HEK) 293 were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin-amphotericin (PSA). The HEK 293 cells were plated at a concentration of approximately 400,000 cells per well in 6 well plates containing poly-L-lysine (PLL) coated glass cover slips for transfection 24 hours later. Each well was transfected with a total of 3µg of cDNA of the antigen of interest using polyethylenimine and glucose. A plasmid encoding for green fluorescent protein (EGFP) was co-transfected to allow visualisation of cell uptake of the cDNA in the NMDAR-Ab CBA. After 16 hours the media was replaced by freshly supplemented DMEM. In the NMDAR transfected cell plates 500µM of ketamine was added to prevent cell death due to activation of receptors by glutamate in the media.*

*48 hours post transfection cover slips containing live antigenic protein expressing HEK cells were incubated in 24 well plates with patient sera at a 1:20 or 1:100 dilution with wash buffer (DMEM supplemented with 1% bovine serum albumin and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) for 60 minutes. Cells were then washed and fixed in 3% formaldehyde for 10 minutes. Alexa Fluor 568 anti-human IgG was applied as a secondary antibody for 45 minutes at room*

temperature. The cells were then washed again and mounted using aqueous mounting medium containing a 1:1000 dilution of 4',6-diamidino-2-phenylindole (DAPI) as a nuclear counter-stain. The mounted cover slips were visualised on a fluorescence microscope by two independent observers and binding was scored on a scale from 0-4 where 0 is “no-binding” and 4 is “strong binding”(Irani et al., 2010a). Examples of binding patterns and scoring for the CASPR2 assay are shown in Figure 2.2.

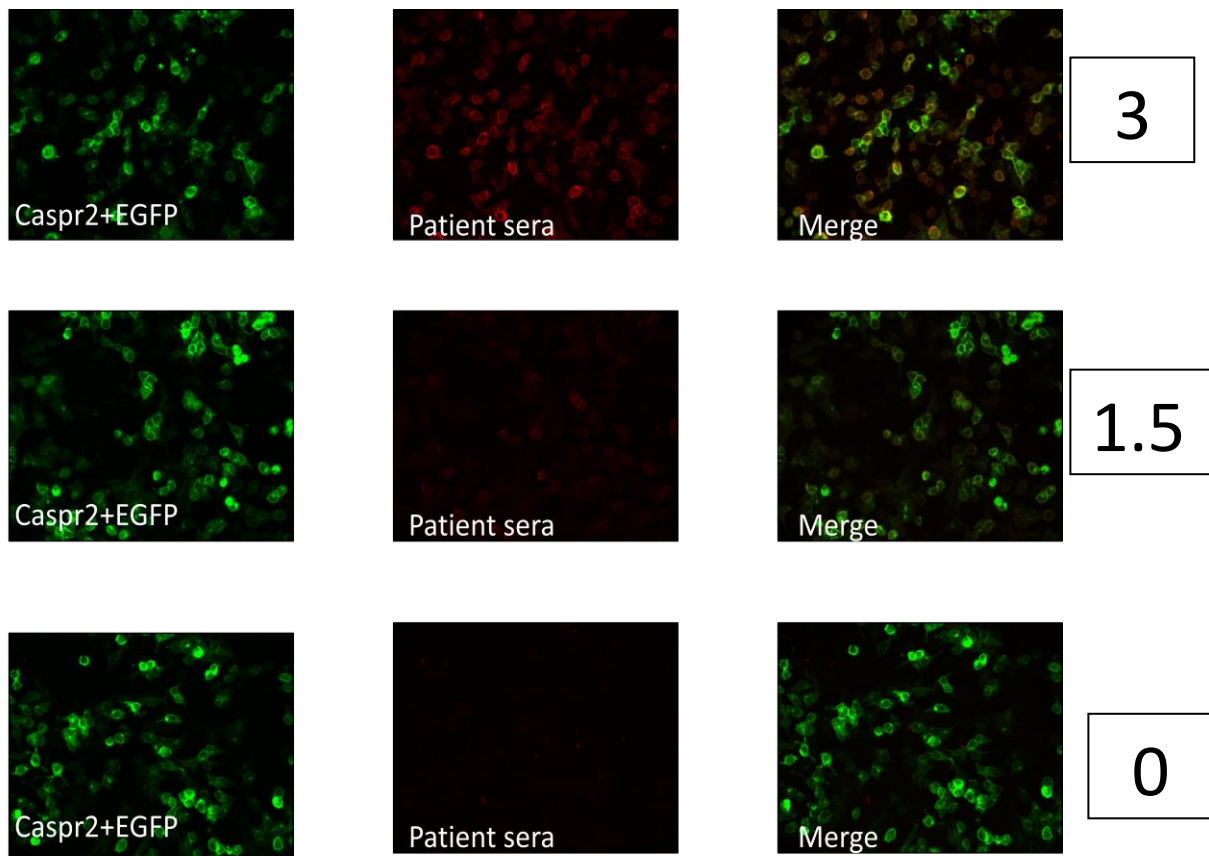
#### **2.4.5.1.2 Radioimmunoprecipitation assay (RIA)**

To determine the VGKC-complex antibodies a radioimmunoprecipitation assay was used as previously described (Hart et al., 1997, Vincent et al., 2004). The extract containing VGKC-complexes was prepared by solubilising whole rabbit brain membranes using 2% digitonin in DTX-buffer (100mM NaCl, 20mM Tris, 5mM KCl adjusted to pH 7.1) at 37°C for 20 minutes and then centrifuging at 13,000g for 15 min at room temperature. The supernatant was harvested, diluted 1:2 with PTX (0.02M phosphate buffer and 0.1% Triton X100, pH 7.2) and incubated with I<sup>125</sup>-αDTX (dendrotoxin). This extract was further diluted with DTX-buffer to give 50,000 counts per minute (cpm) per 50µl or 1 million cpm per ml. The extract was incubated at 4°C until use.

50µl of a 1 in 10 dilution of patient serum was incubated with 50µl of I<sup>125</sup>-αDTX-labelled extract overnight at 4°C. Secondary anti-human antibody was added and incubated at room temperature for 1 hour and 20 minutes. 500µl of PTX was added and samples spun at 13,000g for 5 minutes to form the pellet. Samples were washed immediately twice with 500µl of PTX. The final wash was removed before counting the radioactivity of the formed pellets on the gamma counter. The level of radioactivity remaining in the tube is proportional to the antibody level in the sample.

To try and negate the effects of non-specific binding a ‘hot-cold’ assay was also performed on the samples. This assay subtracted non-specific surface-bound <sup>125</sup>I-αDTX from total surface-bound <sup>125</sup>I-αDTX to estimate specific surface-bound <sup>125</sup>I-αDTX. To achieve this, patient samples were incubated with unlabelled DTX to saturate binding of VGKC-complex receptors before addition of labelled extract as above.

For the GAD antibody RIA,  $I^{125}$ -GAD was used. 50 $\mu$ l of a 1 in 10 dilution of patient serum was incubated with 50 $\mu$ l  $I^{125}$ -GAD overnight at 4 $^{\circ}$ C. Secondary anti-human antibody was added and incubated at room temperature for 1 hour. 500 $\mu$ l of PTX was added and samples spun at 11,000g for 5 minutes to form the pellet. Samples were washed immediately three times with 500 $\mu$ l of PTX. The final wash was removed before counting as above.



*Figure 2.2 Scoring of the cell-based assay, using CASPR2 as an example*

*(courtesy of Sukhvir Wright, John Radcliffe Hospital, Oxford, UK). Left column is EGFP, centre column is human IgG after incubation and right column is overlay. The top row shows binding score 3, the middle row shows binding score 1.5 and the bottom row shows binding score 0.*

#### **2.4.5.2 Creating a normal reference range for VGKC-complex antibody in children**

The routine laboratory cutoff for VGKC Ab is 100 pM, which is the adult healthy control mean + 3 SD. The mean VGKC Ab in our paediatric healthy control group (15 out of 69 controls) was 18 pM (SD 26, mean + 3 SD = 96). As our control mean +3SD was 96 pM, we therefore used the established cut-off of 100 pM to define positive cases.

#### **2.4.6 Statistics**

Fisher's exact nonparametric 2 x 2 test was used to compare categorical data and calculate P values.

### **2.5 Results**

Four of 10 patients with encephalitis and status epilepticus had a positive VGKC Ab titre (>100 pM) compared with only 1/69 of the paediatric control group (mean 20 pM, SD 32,  $p < 0.001$ , 95% confidence interval). The positive control (VGKC Ab 173 pM) had NMDAR encephalitis with refractory clinical seizures. The 10 encephalitis/SE patients were all negative for antibodies against LGI-1, CASPR2, NMDAR, GAD, and GlyR.

The clinical features of the VGKC Ab-positive patients are presented in Table 2-2 (1 male, age range 1–14 years, mean 9 years). All four patients were normal before the acute encephalitis illness. All had SE at presentation. The duration of SE was 30–60 minutes in one patient, 60 minutes–24 hours in one patient, and longer than 24 hours in 2 patients. Patients had ongoing refractory seizure clusters for 5–20 days with up to 15 seizure clusters per day (Table 2-2). In addition to seizures, all patients had encephalopathy and behavioural or cognitive alteration during the acute illness. The four patients had a mean hospital admission of 20 days (range 7–28 days), and all required admission to intensive care for a mean of 7 days (range 2–18 days). The CSF was abnormal in all four VGKC-positive patients with mild pleocytosis (n = 4) and elevated CSF protein (n = 2). CSF polymerase chain reaction (PCR) for herpes simplex virus and enterovirus was negative in all three patients tested. Serology for neurotropic infectious agents was negative including: mycoplasma pneumoniae (n=3), enterovirus (n=3), influenza (n=2), adenovirus (n=2), Epstein-Barr virus (n=2), herpes simplex virus (n=1),

human herpes virus 6 (n=1) and cytomegalovirus (n=1). ANA was elevated at 1:640 in 2 patients (case 1 and 4) but resolved on follow-up. One patient (case 1) had hyponatremia (Na nadir 126 mmol/L). Brain MRI showed abnormalities in 2 cases (Table 2-2). EEG showed slowing in all four patients, generalised in two and focal (temporal) in two. Epileptic activity or electrical seizures were seen in one patient and were of focal (temporal) onset.

For comparison the 6 patients who tested negative for the VGKC Abs are presented in Table 2-3 (3 males, age range 1-11 years, mean 6.3). Two of these patients had pre-existing mild developmental delay/ learning difficulty but no neurologic diagnosis. They all had convulsive status epilepticus which was focal in two and generalised in four. The duration of SE was 30–60 minutes in one patient, longer than 24 hours in 3 patients and difficult to determine in 2 patients. All 6 patients had encephalopathy and behavioural or cognitive alteration during the acute illness in addition to seizures. The 6 patients had a mean hospital admission of 17.6 days (range 6–36 days), and 5 required admission to intensive care for a mean of 8.3 days (range 2–25 days).

In order to determine if there was a difference between VGKC Ab positive and negative patients, we compared the clinical and investigation features of the two groups. We found no significant difference between the positive and negative groups in terms of demographics, clinical presentations and investigation results, although the numbers are small (Table 2-4). It was interesting to note the outcome for the positive patients was poorer than for the negative ones. On follow up for an average of 40 months for the positive group and 11 months for the negative group, all four patients in the positive group had long term consequences: three had ongoing epilepsy, two had significant cognitive impairment, one had mild cognitive impairment and one had psychiatric disturbance. As for the negative group; four patients out of six showed no new deficit (two had complete recovery and two had no worsening of their pre-existing learning difficulty/ developmental delay, one patient had cognitive impairment and one patient died as a result of gastrointestinal complications (bowel perforation; thought to be secondary to barbiturates or ischaemia) . None of the negative patients had ongoing epilepsy or psychiatric disturbance. The difference in epilepsy outcome between the two groups was statistically significant (P value 0.03) see Table 2-4.

*Table 2-2 Encephalitis and status epilepticus patients with positive VGKC Abs*

| <b>Case</b> | <b>Age, sex</b> | <b>Predominant seizure type, and course</b>               | <b>Other clinical features</b>   | <b>CSF microscopy and protein</b>    | <b>EEG</b>   | <b>MRI</b>  | <b>VGKC Ab (pM)</b> | <b>Outcome (length of follow up in months)</b>                              |
|-------------|-----------------|---|--|--------------------------------------|--|---|---------------------|---|
| 1           | 1F              | Generalised Seizure cluster (5 days)                      | Encephalopathy<br>Behavioural alteration<br>Fever  | 9cells/mm <sup>3</sup><br>0.24 g/dl  | Left temporal slowing  | Normal *  | 207                 | Temporal lobe epilepsy<br>Cognitive impairment (66)                         |
| 2           | 9F              | Secondary generalised Seizure cluster (20 days)           | Encephalopathy<br>Behaviour alteration<br>Fever  | 11cells/mm <sup>3</sup><br>0.55 g/dl | Generalised with dominant right temporal slowing                             | Subcortical hyperintensities (bifrontal, left parietal) | 107                 | Temporal lobe epilepsy<br>Cognitive impairment<br>Psychiatric disorder (54) |
| 3           | 12M             | Generalised Seizure cluster (4 days)                      | Encephalopathy<br>Cognitive and behaviour alteration<br>Vomiting<br>Respiratory symptoms | 25cells/mm <sup>3</sup><br>0.52 g/dl | Generalised slowing  | Generalised mild cerebral oedema                        | 214                 | Mild cognitive impairment (17)  |
| 4**         | 14F             | Secondary generalised and focal Seizure cluster (16 days) | Encephalopathy<br>Cognitive and behaviour alteration<br>Fever                            | 6 cells/mm <sup>3</sup><br>0.21g/dl  | Generalised slowing, bitemporal epileptic discharges and electrical seizures | Normal  | 640                 | Seizure recurrence after 2 years seizure freedom (38)                       |

\* Patient had mesial temporal sclerosis on follow up imaging

\*\* Index case



*Table 2-3 Encephalitis and status epilepticus patients with negative VGKC Abs*

| <b>Age, sex</b> | <b>Predominant seizure type, and course</b> | <b>Other clinical features</b>                                      | <b>CSF microscopy and protein</b>    | <b>EEG</b>  | <b>MRI</b>   | <b>Outcome ( length of follow up in months)</b> |
|-----------------|---|---|--------------------------------------|---|--|---|
| 1F              | Generalised, ? Continuous (24 hours?)       | Encephalopathy<br>Behavioural alteration<br>Fever                   | 0cells/mm <sup>3</sup><br>0.24 g/dl  | Generalised slowing, posterior epileptic discharges           | Diffusion restriction in deep WM.<br>Cerebral atrophy at 2 weeks FU  | Developmental delay (pre-existing) (5)          |
| 3F              | Focal<br>Seizure clusters (? duration)      | Encephalopathy<br>Behaviour alteration<br>Fever                     | 11cells/mm <sup>3</sup><br>0.55 g/dl | Generalised slowing, frontal epileptic discharges             | Normal   | Isolated event (12)                             |
| 5M              | Generalised, Seizure clusters (60 min)      | Encephalopathy<br>Cognitive alteration<br>Fever                     | 25cells/mm <sup>3</sup><br>0.52 g/dl | Generalised slowing with right side predominance              | Diffuse leptomenigeal enhancement, brain oedema  | Isolated event (12)                             |
| 9M              | Generalised, Seizure clusters (? duration)  | Encephalopathy<br>Cognitive and behavioural alteration<br>Diarrhoea | 8 cells/mm <sup>3</sup><br>0.46g/dl  | Generalised slowing   | Initial: Diffuse leptomenigeal enhancement. Subsequent: patchy diffusion restriction. ? necrotising encephalitis | Death (1)                                       |
| 10F             | Generalised, Continuous (? duration)        | Encephalopathy<br>Fever   | 6 cells/mm <sup>3</sup><br>0.21g/dl  | Generalised slowing   | left parieto-temporo-occipital swelling of gyri, leptomenigeal enhancement                                       | Learning difficulty (pre-existing) (20)         |
| 11M             | Focal, Seizure clusters (?days)             | Encephalopathy Cognitive and behavioural alteration<br>Fever        | 46cells/mm <sup>3</sup><br>0.32g/dl  | Generalised slowing, left frontotemporal epileptic discharges | Mild cerebral atrophy, increased signal in the left thalamus, mild meningeal enhancement                         | Cognitive impairment (18)                       |

*Table 2-4 Comparison of VGKC Ab negative and positive encephalitis patients*

| <b>Characteristic</b>                                | <b>VGKC Ab negative patients n=6 (%)</b>  | <b>VGKC Ab positive patients n=4 (%)</b> | <b>P value</b> |
|--|---|--|----------------|
| Mean age (y)   | 6.5                                       | 9  | -              |
| Sex (F)  | 3/6 (50)                                  | 3/4 (75)                                 | 0.57           |
| Fever  | 5/6 (83.33)                               | 3/4 (75)                                 | 1.0            |
| Seizure cluster                                      | 4/6 (66.66)                               | 4/4 (100)                                | 0.47           |
| Generalised seizures                                 | 4/6 (66.66)                               | 2/4 (50)                                 | 1.0            |
| Secondary generalised seizures                       | 0/6 (0)                                   | 2/4 (50)                                 | 0.13           |
| Focal seizures                                       | 2/6 (33.33)                               | 2/4 (50)                                 | 1.0            |
| Encephalopathy                                       | 6/6 (100)                                 | 4/4 (100)                                | 1.0            |
| Cognitive or behavioural alteration in acute illness | 5/6 (83.33)                               | 4/4 (100)                                | 1.0            |
| Length of hospital stay-mean (range) in days         | 17.6 (6–36 )                              | 20 (7–28)                                | -              |
| PICU admission                                       | 5/6 (83.33)                               | 4/4 (100)                                | 1.0            |
| Length of PICU stay-mean (range) in days             | 8.3 ( 2–25)                               | 7 ( 2–18)                                | -              |
| CSF pleocytosis                                      | 5/6 (83.33)                               | 3/4 (75)                                 | 1.0            |
| CSF cells mean (cells/mm <sup>3</sup> )              | 16  | 12.75                                    | -              |
| High protein (>0.40g/dl)                             | 3/6 (50)                                  | 2/4 (50)                                 | 1.0            |
| CSF protein mean (g/dl)                              | 0.38                                      | 0.38                                     | -              |
| EEG slow   | 6/6 (100)                                 | 4/4 (100)                                | 1.0            |
| EEG epileptic  | 3/6 (50)                                  | 1/4 (25)                                 | 0.57           |
| EEG focal  | 4/6 (75)<br>(post/frontal/frontotemporal) | 3/4 (75)<br>(temporal)                   | 1.0            |
| MRI abnormal   | 5/6 (83.33)                               | 2/4 (50)                                 | 0.5            |
| Outcome:   |   |  |                |
| Length of follow up-mean (range) in months           | 11.33 (1-20)                              | 43.74 (17-66)                            | -              |

|                                  |             |          |              |
|----------------------------------|-------------|----------|--------------|
| Isolated event/complete recovery | 4/6 (66.66) | 0/4 (0)  | 0.076        |
| Ongoing epilepsy                 | 0/6 (0)     | 3/4 (75) | <b>0.033</b> |
| Cognitive impairment             | 1/6 (16.66) | 3/4 (75) | 0.19         |
| Behavioural/psych disturbance    | 0/6 (0)     | 1/4 (25) | 0.4          |
| Death                            | 1/6 (16.66) | 0/4 (0)  | 1.0          |

### 2.5.1 Case 2 presentation

We present here the case history of another VGKC Abs positive patient (case 3 in Table 2-2) for comparison and to get more insight into the details of the clinical phenotype of VGKC complex Ab associated encephalitis in children.

A 12 year old Caucasian male had a past medical history of repair of bilateral inguinal hernia and undescended testicles in the first year of life and recurrent ear infection in the first 3 years of life, but no other significant illnesses. He presented with a subacute illness over 7-10 days of nausea and vomiting initially, then rhinorrhea, sore throat and cough in addition to being irritable and not himself. This was followed by headache that got worse the day prior to his admission. On the morning of admission he was found by his mother on the floor after hearing noises. He was moaning, nonresponsive, had jerky movements of all limbs, stiffness all over his body and incontinent of urine. The duration of the seizure was unknown but longer than 20 minute. He had reduced level of consciousness on ambulance arrival and was irritable, however seizure activity has stopped. At the local hospital he was described to be irritable with reduced level of consciousness and a Glasgow Coma Score (GCS) of 8/15. He had no fever and no definite focal findings on neurological examination. He was commenced on acyclovir and cefotaxime for suspected meningoencephalitis. He was electively intubated due to reduced level of consciousness and in preparation for the CT scan and for the transfer to our tertiary hospital. A head CT scan was performed and found no abnormality. He was transferred to our PICU and had a low grade fever of 38 C. He became more responsive and was extubated. A few hours later he had episodes of confusion and frequent jerking movements that were

thought to be focal seizures. He was given midazolam boluses, reintubated and commenced on midazolam infusion. Phenytoin was commenced.

EEG showed diffuse slowing at 1-2 Hz with superimposed continuous fast activity of 14-16 Hz (likely related to benzodiazepines treatment) with no epileptic activity. A repeat CT scan of the brain showed poor differentiation of white and grey matter indicating cerebral oedema. MRI brain was normal.

CSF examination revealed 5 polymorphonuclear and 20 mononuclear cells/ mm<sup>3</sup>, normal glucose and elevated protein at 0.52 g/dl. CSF PCR for herpes simplex virus and enterovirus were negative.

Hyponatraemia was not a feature of the biochemistry. Serology for mycoplasma, cytomegalovirus, Epstein Barr virus, adenovirus, enterovirus and influenza were negative. Nasopharyngeal aspirate was negative for respiratory viruses and stool microscopy and culture was negative.

During his four day stay in PICU he continued to have clusters of seizures that were described as generalised lasting 2-4 minutes each. He was maintained on phenytoin as well as on midazolam infusion. He also developed episodes of hypotension requiring fluid boluses, alternating with episodes of hypertension not requiring specific treatment. He had episodes of agitation, confusion and thrashing around while maintained on midazolam and morphine infusions that were treated with extra sedation including chloral hydrate. On day four of his admission he was discharged to the ward. He made slow but steady clinical improvement and was discharged on day 10.

On review at one month and one year after his illness he was seizure free and maintained good health. Parents raised concerns related to some personality changes and loss of some “autobiographical” memories for events from childhood that he previously recalled. Formal Neuropsychological assessment was conducted 17 months after his illness and revealed average intellectual abilities, but difficulties in keeping track of mental operation and more demanding problem solving.

## **2.6 Discussion**

In this retrospective study of a small cohort of children with unexplained encephalitis and status epilepticus we found that four out of 10 patients were positive for the VGKC-complex antibodies.

This suggests that VGKC Ab associated encephalitis/ encephalopathy exists in children and may be an important cause of paediatric encephalitis complicated by seizures, cognitive and behavioural alteration.

We found no significant difference between the positive cases and the negative cases in terms of demographics and clinical presentation. The clinical presentation in all 10 patients was predominantly that of a subacute presentation of new onset seizures, status epilepticus, encephalopathy, behavioural and or cognitive alteration, with or without preceding infection. Seizures were generalised, focal, or secondary generalised. The seizure clusters occurred over days and seizures were generally challenging to treat. The CSF was mildly abnormal with variable degrees of pleocytosis and elevated protein. CSF viral PCR testing was negative when performed and serology for neurotropic agents was negative. MRI in all was either normal or showed non-specific abnormalities (see Tables 2-2, 2-3 above). All patients showed a diffusely slow EEG. Interestingly for the positive patients when focal features were present in the EEG (focal slowing, epileptic activity or electric seizures) they were predominantly in the temporal region. Temporal lobe abnormalities are well described in adults with this syndrome (Vincent et al., 2004).

The VGKC-complex antibody positive cases had poorer outcome compared to negative cases. All the positive patients had residual deficits including: ongoing epilepsy (temporal lobe epilepsy) in two, recurrence of seizures in one, cognitive impairment in three and psychiatric disturbance in one case. In comparison none of the negative patients had ongoing epilepsy or psychiatric disturbance, although one had residual cognitive impairment. The positive cases were followed up for a longer duration (mean 43.7 range 17-66 months) compared to the negative cases (mean 11.3 range 1-20 months). It was interesting to note that the severity of the presenting illness did not predict outcome in these patients. The poorer outcome in our VGKC-complex Abs positive cases might indicate that this form of encephalitis have a worse outcome if untreated. None of the positive cases received immunotherapy early and one (index case) received a short course of immunotherapy late in the course of her illness (15 months after onset). It is possible that this was late and insufficient immunotherapy for this child as she has had recurrence of her epilepsy.

Our four positive paediatric cases had similarities in their clinical presentation to adults with VGKC-complex Ab associated LE as they had seizures, encephalopathy, behavioural and cognitive alteration. Our paediatric patients had worse seizures than the seizures described in adults but this may be due to our selection process that included patients with encephalitis and status epilepticus. The seizures described in our paediatric patients were not similar in semiology to the distinctive faciobrachial dystonic seizures described in some adults with LGII antibodies (Irani et al., 2011b), although video recording of seizures was only available for review in one of them (index case). One of our patients had hyponatremia which is found commonly in adult patients (Vincent et al., 2004). The CSF in our paediatric patients was normal or mildly abnormal. This is similar to findings in adults with VGKC-complex associated LE (Thieben et al., 2004, Vincent et al., 2004, Jarius et al., 2008). The Brain MRI in our children was normal or non specific unlike adults described with the syndrome. Eight out of 10 patients described by Vincent et al, 2004 had mesial temporal abnormalities including high signal initially and atrophy subsequently (Vincent et al., 2004). One of our paediatric patients had mesial temporal sclerosis on follow up, a finding that is similar to what has been described in a 13 year old girl following LE associated with VGKC Abs (Kroll-Seger et al., 2009). Our patients' EEGs and seizures semiology were of temporal lobe predominance. Two of our cases had ongoing temporal lobe epilepsy (TLE). This supports the hypothesis that VGKC-complex Ab has predilection to the temporal lobe structures (as part of the limbic system). The evolution from limbic encephalitis to temporal lobe epilepsy and mesial temporal sclerosis has been described in adults (Bien et al., 2007b). None of our patients had a coexisting immune disease but two had transient elevation of ANA. Elevated ANA have been previously described in adult patients with positive VGKC Abs (Vincent et al., 2004).

In our patients' sera we did not identify binding to LGII or CASPR 2. It is likely that these VGKC complex antibodies bind to other proteins in the VGKC-complex that are yet to be identified, or to the VGKC itself. Further studies are required to examine whether antibodies to LGII, CASPR 2 or other proteins tightly complexed with the VGKC are present in children with autoimmune brain disease.

Using the definition of positivity as per Haberlandt et al (normal <100pM), low positive (100–150 pM), positive (150–400 pM) and high positive (>400 pM) (Haberlandt et al., 2011) one of our four

positive cases (case 2, Table 2-2) had a low positive titre of VGKC antibodies (107 pM). Lower titres might be associated with lower certainty about the significance of these antibodies and their pathologic role. Positive antibodies might be a secondary phenomenon in these patients.

One of our paediatric control cases was positive for VGKC- complex Abs and this patient had the clinical features of NMDAR encephalitis and refractory seizures. This patient was positive for both VGKC and NMDAR antibodies. The finding of more than one autoantibody type has been described in adult patient with autoimmune limbic encephalitis (Pellkofer et al., 2010), and is also now increasingly recognised (Irani et al., 2010a, Haberlandt et al., 2011).

The VGKC-complex Ab LE in adults is thought to be immune-responsive and have a better outcome when compared to the para-neoplastic form of LE. While this comparison is difficult to conduct in children as para-neoplastic LE is rare in children, our cohort showed that VGKC-complex Ab associated encephalitis in children generally has a poor outcome. This could be due to the fact that the diagnoses were made retrospectively and none of the patients received early adequate immunotherapy. In adults the syndrome is now well recognised and appropriate treatment regimens are still under evaluation. In children the situation is different and treatment is likely to be delayed as this form of encephalitis is not well recognised. Testing for VGKC-complex Abs is only done in a few specialised laboratories in the world and this can delay the results. However commercial companies are now producing kits that might be used locally and reduce the diagnostic delay.

The findings in our study and in the other reports from paediatric cases (Dhamija et al., 2011, Haberlandt et al., 2011) have helped change our clinical practice. At present in our hospital we try to identify the patients with autoimmune encephalitis early. We send their sera for testing however we commence immunotherapy while results are still pending. As there are no guidelines available for children we have adopted an intensive treatment regimen consisting of intravenous methyl prednisolone at 30mg/kg/day for 3 days, as well as intravenous immunoglobulin of 2 g/kg, followed by oral prednisolone tapering treatment over weeks or months depending on severity. The intravenous treatment with immunoglobulin and/or methyl prednisolone can be repeated after a few weeks if

adequate improvement is not achieved. Alternatively plasma exchange can be considered or second line therapy (discussed later, see Chapter 4). There is no consistent protocol and decision-making is left to the discretion of the involved clinician. We suspect that early and adequate treatment is necessary to achieve the best possible outcome. Any delay in treatment may result in irreversible damage.

We conclude that VGKC-complex Ab associated encephalopathy exists in children as it does in adults and may be an important cause for new onset seizures, encephalopathy, behavioural and cognitive alteration, psychiatric disturbance, temporal lobe epilepsy and mesial temporal sclerosis. We recognise the need for larger studies in children to help further understand the role of VGKC-complex antibodies in children.



# **Chapter 3 Neuronal antibodies in children with new onset seizures classified according to the revised ILAE classification 2010**

## **Acknowledgment**

**This chapter is a detailed and a slightly modified version of the published paper (Suleiman et al., 2013b) , see Appendix 6.**

## **3.1 Introduction**

The role of autoantibodies has been increasingly recognised in the pathogenesis of epilepsy. This includes antibodies against neuronal surface antigens such as VGKC-complex, LGI1, CASPR2, NMDAR, GABA<sub>B</sub>R, and AMPAR as well as intracellular antigens such as GAD. The association between these antibodies and seizures is best described in patients with limbic encephalitis who often have temporal lobe seizures. However epilepsies of autoimmune basis have been described in the absence of limbic encephalitis (Peltola et al., 2000, McKnight et al., 2005, Majoie et al., 2006, Irani et al., 2008, Niehusmann et al., 2009, Barajas et al., 2010, Quek et al., 2012). Currently “autoimmune epilepsy” is becoming a recognised term that is used in patients with epilepsy who have positive neuronal antibodies (Irani et al., 2011a, Vincent et al., 2011b, Quek et al., 2012).

McKnight et al found positive VGKC antibodies in 16 out of 139 patients with epilepsy that they studied (11%) and high level GAD antibodies (defined as more than 1000 U) in three out of the 139 patients (2.1%) (McKnight et al., 2005). VGKC antibody positive patients were older at age of seizure onset and often had accompanying acute or subacute encephalopathy, whereas GAD antibodies positive patients were younger at age of seizure onset and had chronic drug-resistant epilepsy.

Faciobrachial dystonic seizures (FBDS) are seizures with distinctive semiology that has been described in patients with LGI1 antibodies that sometimes precedes limbic encephalitis (Irani et al., 2008, Irani et al., 2011b). These are frequent, brief dystonic seizures that predominantly affected the

arm and ipsilateral face. It is thought that recognizing and treating these seizures with immunotherapy might prevent progression to encephalopathy and cognitive impairment.

A recent study found neuronal antibodies in 11% of two adult patients cohorts with new and established epilepsy including VGKC complex proteins (5%), glycine receptor (3%), GAD (1.7) and NMDA receptor (1.7%) (Brenner et al., 2013). There was no significant difference between patients with newly diagnosed and established epilepsy. However positive antibody were found more in patients with focal epilepsy of unknown cause than in those with structural/metabolic focal epilepsy (14.8% vs. 6.3%;  $p < 0.02$ ).

In children fewer reports are available regarding the association of neuronal antibodies and epilepsy. NMDAR encephalitis occurs in children and focal or generalised seizures are an important feature. VGKC-complex antibodies have been reported in children with limbic encephalitis (Kroll-Seger et al., 2009, Haberlandt et al., 2011) and in some children with status epilepticus in the context of unexplained encephalitis (Suleiman et al., 2011a). There are a few recent paediatric reports that describe VGKC antibodies in children with epilepsy in the absence of encephalitis including a child with epileptic encephalopathy and epileptic spasms (Suleiman et al., 2011b) and two children with symptomatic generalised epilepsy (Dhamija et al., 2011).

Seizures and epilepsy have many types and causes and accurate description and classification is important on clinical and research levels. The ILAE classification system is widely used and has been revised a few times since its implementation in 1969 to meet the ongoing developments in the understanding of epilepsy and its causes. The classification of seizures and epilepsies involves three major areas or "axes" including seizure type and semiology, epilepsy syndrome and epilepsy aetiology (Engel, 2001). The latest ILAE report in 2010 presented a revision of terminology and concepts and approaches for classification of seizures, epilepsy syndromes and epilepsy aetiology (Berg et al., 2010). The new report proposed using a simplified meaningful approach and suggested changing a few existing terms including the replacement of the terms "idiopathic, symptomatic,

and cryptogenic” with “genetic, structural/metabolic and unknown cause” when describing aetiology (Berg et al., 2010).

## **3.2 Hypothesis and aims**

There have been no large paediatric cohort studies to examine the role of neuronal antibodies in children with epilepsy. We suspect that a proportion of children with epilepsy have an autoimmune cause of their epilepsy. In these patients early immune therapy might improve their outcome.

We aimed to prospectively recruit children with new onset seizures, record their demographic and clinical features, classify their seizures and epilepsies according to the ILAE classification system and test them for neuronal antibodies. This study aimed to determine the proportion of epilepsy with a putative autoimmune aetiology. In addition, this study examined the utility of the new ILAE classification.

## **3.3 Methods**

This was a prospective study that recruited children with new onset seizures presenting to the Children’s Hospital at Westmead from September 2009 to November 2011.

### **3.1.1 Power calculation and sample size**

Based upon the results of the McKnight study where 11% of adult patients were positive for VGKC antibodies we assumed that 11% of our patients would be positive.

We performed a power calculation assuming that 11% of patients and 1% of controls will have autoantibodies. We expected to be able to recruit more patients than controls. Using significance of  $p < 0.05$ , and power of 80% (standard), the number of patients required was 115 and the number of controls was 58.

### **3.3.2 Ethics approval**

Ethics approval from the Ethics Committee at the Children Hospital at Westmead was sought. The process included filling an online application form with detailed information about the study and its methodology. This was reviewed and approval was obtained in August 2009 (for ethics application and associated information see Appendix 3).

In addition clinicians involved in the management of patients with new onset seizures were informed about the study including paediatric neurologists and general paediatricians at the hospital who agreed to recruit their consenting patients.

### **3.3.3 Patient recruitment**

We recruited consenting patients with the following inclusion criteria:

- Age 2 months to 16 years
- New onset seizures of any types or aetiology (except those excluded, see below)
- Serum collected within six months after seizure onset. We think that testing for antibodies around the time of onset of seizures provides the best chance to understand the relevance of these antibodies
- Patients were inpatients or outpatients at The Children's Hospital at Westmead

A seizure was defined based on the clinical history of an episode that was suggestive of a seizure.

A clinical seizure was defined as per by ILAE Guidelines: “the clinical manifestation consists of sudden and transitory abnormal phenomena which may include alterations of consciousness, motor, sensory, autonomic, or psychic events, perceived by the patient or an observer”

(Commission On et al., 1993). Patients were included if they had a single or multiple seizures as determined by the treating clinician. This included patients with febrile seizures.

The following patients were excluded from the study:

- Neonates (0-60 days) as many of the causes for their seizures are age specific and have different aetiological factors.
- Patients who had a clear cause for their seizures at onset including bacterial meningitis, strokes, traumatic brain injury and brain tumors. Patients with malformation of cortical development or neurocutaneous syndromes were not excluded as dual pathology is possible and recognised. For example, cortical dysplasia has been described in patients with Rasmussen encephalitis, which is thought to be immune mediated (Palmer et al., 1999).
- Patients whose serum sample was collected more than 6 months from onset of first seizure.
- Patients with non epileptic paroxysmal events. Distinguishing seizures and epileptic events from non epileptic events including pseudo-seizures, syncope, sleep disorders and others was based on the clinical manifestations. Patients who had clinical events suggestive of seizures but when captured on EEG were consistent with alternate diagnosis (such as non epileptic events) were excluded.

Patients were identified by the primary investigator (JS) through reviewing the inpatient list on a daily basis, and collaboration with medical staff in the medical and neurology teams at the hospital. The recruited patients were from a tertiary referral hospital, which might have caused bias in the types and severity of the patients' epilepsies. Patients were more likely to be seen and recruited by the primary investigator if they were inpatients rather than outpatients, and if they had a longer hospital admission. Patient recruitment took place over 2 years. 114 patients were recruited of whom 104 patients were seen by a paediatric neurologist during the course of their illness either as primary physician or consultant. The remaining 10 patients were managed by a general paediatrician. 16 patients were transferred from another hospital to our hospital for tertiary care.

### **3.3.4 Consenting process**

Information sheets about the study were created for both parents and young patients and approved by the Ethics Committee at the Children's Hospital at Westmead (see Appendix 3). These letters explained background information as well as the purpose of the study in lay terms and also explained

the requirement of blood collection for the study. Patients and families were informed that they have the full choice to enroll in the study and that the results of this study were not going to change their current management.

The ethics committee approved consent forms were signed by the parents and patients who were older than 14 years (see Appendix 3).

Most patients were approached while in the hospital (wards or clinics) and given information sheets and consent forms. Signed consents were then either collected in person or posted back to the investigators. Patients who were referred by other medical staff and were not seen by the primary investigator were sent information sheets and consent forms by mail with reply paid envelopes to be returned (free of charge) to the investigators.

### **3.3.5 Serum collection**

A serum sample was collected for all consenting patients. The serum was collected either for a clinically indicated test that required a serum sample (for example serology for suspected infective agents), or collected in conjunction with other indicated tests such as drug levels. None of the children enrolled in this study were exposed to blood sampling merely to collect serum for this study.

The serum sample had to be collected within 6 months of the onset of seizures. Patients who had serum collected outside this time window were excluded. Most sera were collected during the acute admission associated with the first seizure. If an acute admission sample was not possible, then a blood form was given to the parents to collect serum along with the next blood test required for clinical care (for example at the time of liver function testing after starting sodium valproate). Getting a serum sample within the indicated window (6 months) was a major determinant factor for enrolling patients. Many patients were identified then excluded as a result of being unable to obtain a serum sample, particularly outpatients who were not hospitalized for their seizures. The serum samples were saved in the hospital laboratory. At the end of recruitment phase the serum samples were retrieved,

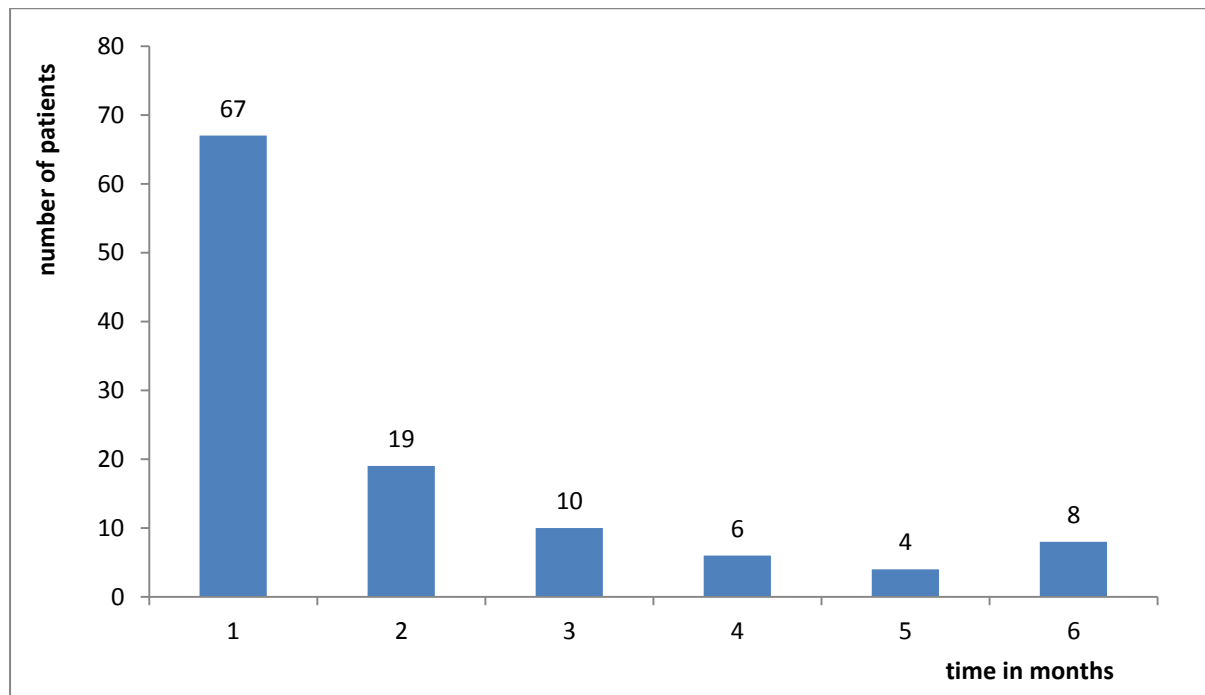
coded and dispatched to our collaborators Beth Lang and Angela Vincent at John Radcliffe Hospital, Oxford to be tested for neuronal antibodies as below.

### **3.3.5.1 Serum samples of the cohort**

Serum sample date and serum sample timing in relation to onset of epilepsy (in days) were reported for each of the 114 patients.

The date of onset of seizures was easy to ascertain if the patient presented to the hospital acutely. In some patients where seizures were more subtle or less severe the seizures might have been occurring for a period of time prior to presentation. The date of onset was determined as best as could be recalled by the parents or carer.

Serum samples were all collected within 6 months (180 days) of seizure onset as per inclusion criteria. Serum collection time range was 0-180 days with a mean of 38.35 and a median of 15.5 days after the first seizure (see Figure 3.1). Sixty seven patients (57.7%) had their serum collected within 1 month and 86 (75.4%) within 2 months (see Figure 3.1).



*Figure 3.1 Serum sample collection timing for total cohort (n=114)*

### **3.3.6 VGKC- complex and other neuronal antibodies assays**

The following antibodies were tested in the 114 patients: VGKC-complex (n=114), NMDAR (n=114), LGI1 (n=113), CASPR2 (n=113), GAD (n=113), Glycine R (n=112), AMPAR (n=112) and contactin-2 (n=112). The minor incomplete testing was due to some samples becoming exhausted. The methods of testing were the same as described in Chapter 2. The antibodies to VGKC-complex and GAD were tested by radioimmunoassay (RIA). VGKC Abs and GAD levels greater than 100 pM and 100 U/ml respectively were considered positive. All other neuronal antibodies were tested by cell based assays (CBA). The CBA were scored on a visual scale 0 (no binding)-4 (strong binding to all transfected cells) by 2 independent observers. Results were considered positive if scoring >1 in NMDAR, LGI1, and CASPR2 assays and >2 in the Gly-R assay. Assays were performed and read by Sukhvir Wright (Oxford) who was blinded to the clinical data, and re-scored by an additional reader.

### **3.3.7 Data Collection**

The prospective nature of the study was mainly to ensure that serum was collected within the window period of 6 months from seizure onset. However the investigators did not perform a separate clinical assessment for the patients unless they were responsible for their care. Hence patients' information was recorded by reviewing the hospital electronic medical recording system including clinical notes from presentation to emergency and admission to hospital, referral letters from other hospitals, hospital discharge summary and clinic letters. Data collected included demographics, previous medical and neurologic history, family history of epilepsy, seizure onset and type, the presence of status, associated clinical features (including encephalopathy, movement disorder, cognitive impairment or inter-current illness), hospital and intensive care admission, investigations including EEG, imaging and CSF, seizure and epilepsy treatments including the use of steroids and/or immunoglobulins, outcome including ongoing epilepsy, drug resistance, developmental delay, cognitive or psychiatric impairment and others (see Appendix 5).

The data collection was performed and completed by the primary investigator (JS) with complete blinding to the result of antibody testing.



### 3.3.7.1 Demographic features of the cohort (n=114)

A total of 114 patients with new onset seizures were included in this study (58 females, mean age at onset 4.39 years (median 2.55 and range 0.13-15.25 years). 51 patients (44.7%) were younger than 2 years (see figure 3.2). This mainly reflects the fact that younger children and infants were more likely to be admitted to the hospital and investigated after their first seizure and therefore more likely to be recruited for this study.

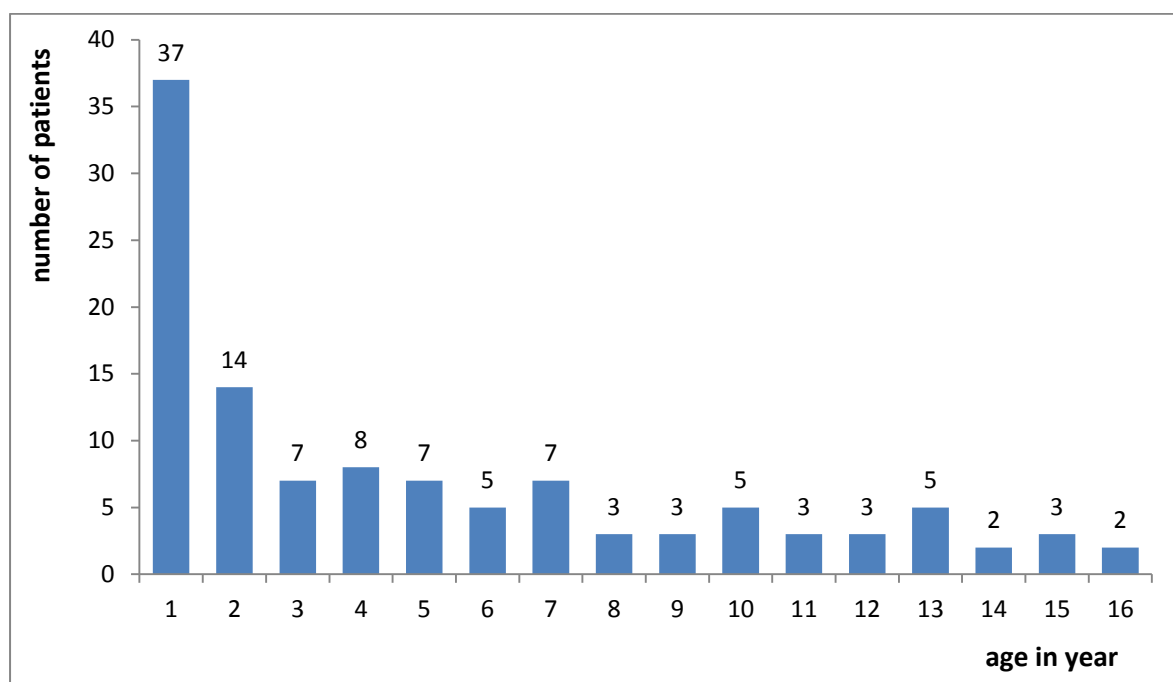


Figure 3.2 Distribution of cohort according to their age (n=114)

### 3.3.7.2 Hospital admission and intensive care treatment

Hospital admission and length of stay as well as admission to the intensive care unit and length of admission were reported as 'surrogate markers' of the severity of illness.

101 out of the 114 patients were admitted to the hospital during their initial presentation or during the first six months after seizure onset. The first hospital stay was used for reporting of duration of hospital admission and intensive care treatment. The mean length of hospital stay was 13.36 days (median 4.5 days, range 1 to 360 days). 18 patients were admitted to the intensive care unit during

their initial presentation or hospitalization. The mean length of intensive care admission was 8.15 days (median 2 days, range 0.8 to 64 days).

### ***3.3.7.3 Clinical features of the cohort***

#### **3.3.7.3.1 Past neurological and medical history**

During data retrieval we recorded the presence of pre-existing:

1. Developmental delay including speech, language, gross or fine motor delay in the young children or learning difficulties in older children.
2. Behavioural or psychiatric impairment including anxiety, depression, attention deficit and hyperactivity.
3. Motor deficits such as hypotonia, spasticity, hemiplegia or other deficits.

These features were considered to be present if they were formally diagnosed previously or reported by the parent or carer, or if they were evident to the treating clinician at the time of assessment for the new onset seizures and known to be longstanding.

Other neurologic or medical diagnoses including that of autoimmune diseases were also reported.

None of the recruited 114 patients had preceding seizures or epilepsy; however on further review of clinical data three patients had possible febrile seizures when younger (9, 6 and 5 years earlier than their current presentation) - we nominated to keep these patients in the cohort.

78 patients had no preexisting neurological or developmental abnormality and 36 had one or more preexisting developmental, motor or psychiatric abnormality including 33 with developmental delay and /or learning difficulty, 14 with motor deficits and four with behavioral or psychiatric disturbance.

22 patients had one or more of the following neurologic diagnoses including: visual or hearing impairment (n=5), chromosomal abnormalities (Trisomy 21, 14q21 deletion, tetrasomy 15q11.2-q13.1, chromosome 7 inversion) (n=5), cerebral palsy (n=3), dysmorphic features and undiagnosed

syndromes (n=2), microcephaly (n=2), microform holoprosencephaly (n=1), neurofibromatosis type 1(n=1), congenital myopathy (n=1), congenital nystagmus (n=1) and propionic acidaemia (n=1).

41 patients had one or more preexisting medical condition, as follows:

- Congenital malformations (n=10): including congenital cataract, choanal atresia, laryngomalacia, trachea-oesophageal fistula, congenital cardiac disease, diaphragmatic hernia, vesicoureteric reflux, undescended testicles, hip dysplasia and skin haemangioma.
- Atopy (n=10): including asthma, eczema, hay fever and food allergies.
- Gastrointestinal (n=5): including Crohn's disease, Hirschsprung disease, chronic constipation, feeding difficulties and celiac disease.
- Autoimmune disorders (n=4): including type 1 diabetes mellitus, Henoch-Schonlein purpura and autoimmune chorioretinitis.
- Premature delivery (n=3).
- Obesity (n=2).
- Endocrine disorders (n=2), including diabetes insipidus and hyperinsulinism.
- Short stature (n=1).
- Recurrent bronchiolitis (n=1).
- Juvenile Myelocytic Leukemia (n=1).

### **3.3.7.3.2 Family history**

A family history of epilepsy or seizures in first-degree relatives was reported. This was determined by reviewing medical records and reported as present when clearly mentioned in the clinical history.

However under reporting is a possibility as this study was not designed to study the genetics of epilepsy.

There was a family history of seizures or epilepsy (including febrile seizures) in first degree relatives in 19 patients.

### 3.3.7.3.3 Presenting seizure characteristics

The seizure semiology at onset was determined from the description available in the medical records, and as reported by the parent, carer or witnesses from clinical staff including nursing and medical staff. The classification system for seizures is evolving and changing hence we used the latest ILAE classification (Berg et al., 2010), in addition to previous ILAE classification reports (Bancaud et al., 1981, Engel, 2001, Engel, 2006). Seizures were classified as follows:

1. Focal when the initial semiology of the seizure was consistent with initial activation of only part of one cerebral hemisphere (ICES 1981) (Bancaud et al., 1981). This includes:
  - a. Focal motor, autonomic or sensory (without impairment of consciousness).
  - b. Focal dyscognitive was defined as a focal seizure that was associated with impairment of consciousness or awareness (Engel, 2006, Berg et al., 2010).
  - c. Focal evolving to a bilateral convulsive seizure (including tonic, clonic or tonic clonic), which was recommended to replace the previous similar term “secondarily generalised seizure” (Berg et al., 2010).
2. Generalised was used when the initial semiology was consistent with involvement of both cerebral hemispheres (ICES 1981).
3. Spasms: Epileptic spasms have been classified as “unknown” in the latest ILAE classification (Berg et al., 2010), hence we kept them in a separate category.

When possible, further characterization of seizure semiology was performed as per the ILAE commission report: Glossary of Descriptive Terminology for Ictal Semiology (Blume et al., 2001), see Table 3-1.

*Table 3-1 Seizure types at presentation: definitions used in the cohort*

*(generated from glossary of descriptive terminology for ictal semiology, Blume 2001)*

|                          |  |
|--------------------------|--|
| Focal                    | “A seizure whose initial semiology is consistent with initial activation of only part of one cerebral hemisphere”  |
| Generalised              | “A seizure whose initial semiology is consistent with more than minimal involvement of both cerebral hemispheres”  |
| Motor                    |  |
| Tonic                    | Sustained contraction in the muscles lasting a few seconds to minutes  |
| Epileptic spasms         | A sudden flexion or extension or combination of both and mainly involving proximal and truncal muscles that is more sustained than a myoclonic jerk but less than a tonic seizure (i.e about 1 second). Limited forms may occur such as head nodding and grimacing. Epileptic spasms often occur in clusters |
| Versive                  | A sustained forced conjugate ocular, cephalic and/or truncal rotation or deviation from midline  |
| Myoclonic                | Sudden, brief, non sustained involuntary single or multiple contractions of muscles or group of muscles (axial, proximal limb, distal limb)  |
| Clonic                   | Myoclonus that is regularly repetitive and involves small muscle groups at 2-3 contractions per second (rhythmic myoclonus)  |
| Tonic clonic             | A sequence consisting of tonic followed by clonic phase  |
| Generalised tonic clonic | Bilateral symmetric tonic contractions then bilateral clonic contractions of muscles   |
| Atonic                   | Sudden loss (or diminution) of muscle tone without apparent preceding myoclonic or tonic even, involving head, trunk, jaw or limb muscles.   |
| Astatic (drop attack)    | Loss of erect posture that result from atonic, myoclonic or tonic mechanism  |
| Automatism               | Repetitive motor activity usually occurring when cognition is impaired   |
| Sensory                  |  |
| Somatosensory            | Tingling, numbness, electric shock feeling, pain, sense of movement or desire to move  |
| Visual                   | Flashing or flickering lights, spots, simple patterns, scotomas or amaurosis   |
| Auditory                 | Buzzing, drumming sounds or single tones   |
| Autonomic                | A sensation consistent with involvement of the autonomic nervous system including cardiovascular, gastrointestinal, vasomotor and thermoregulatory function  |

The presenting seizure(s) in the 114 patients were classified into focal (n=56), generalised (n=42) and spasms (n=16) and are presented in Table 3-2. Impairment of consciousness was determined from commentary made by a witness including parent, carer or clinician, or when other terms which imply impairment of consciousness were used such as “unresponsive”, “confused”, “vague” etc. Sensory phenomena and auras are likely to be under reported in this cohort given the young age of the patients who are unable to describe their auras and seizures. A proportion of generalised and focal seizures were not further classified, as detailed descriptions of seizure semiology were inadequate to characterize them, and we used the term “unclassified” for those seizures.

#### **3.3.7.3.4 Status epilepticus (n=21)**

Status epilepticus was defined as a single seizure lasting more than 30 minutes or a series of seizures without recovery in between the ictal events over a period of at least 30 minutes (Commission On et al., 1993). Twenty one patients had status epilepticus as their initial presentation and all were admitted to the hospital including nine who were admitted to the intensive care unit.

#### **3.3.7.3.5 Early seizure recurrence (n=77)**

Early recurrence of seizures, occurring within 48 hours of first seizure onset occurred in 77 out of the 114 patients.

*Table 3-2 Seizure types at onset for the total cohort (n=114)*

*(classified as per Berg 2010 and Blume 2001)*

| <b>Seizure type</b>              |  | <b>Patients (number)</b> |
|----------------------------------|--|--------------------------|
| <b><i>Focal (n=56)</i></b>       |  |                          |
|                                  | Focal motor                            |                          |
|                                  | Tonic                                  | 2                        |
|                                  | Clonic                                 | 2                        |
|                                  | Myoclonic                              | 2                        |
|                                  | Versive                                | 2                        |
|                                  | Automatism                             | 2                        |
|                                  | Tonic clonic                           | 1                        |
|                                  | Other focal motor                      | 6                        |
|                                  | Focal dyscognitive                     | 12                       |
|                                  | Focal evolving to bilateral convulsive | 8                        |
|                                  | Focal sensory                          |                          |
|                                  | Visual                                 | 3                        |
|                                  | Somatosensory                          | 2                        |
|                                  | Auditory                               | 1                        |
|                                  | Autonomic                              | 1                        |
|                                  | Focal unclassified                     | 10                       |
| <b><i>Generalised (n=42)</i></b> |  |                          |
|                                  | Tonic clonic                           | 28                       |
|                                  | Absence                                | 3                        |
|                                  | Myoclonic                              | 2                        |
|                                  | Clonic                                 | 2                        |
|                                  | Tonic                                  | 3                        |
|                                  | Myoclonic atonic                       | 1                        |
|                                  | Generalised unclassified               | 3                        |
| <b><i>Unknown (n=16)</i></b>     |  |                          |
|                                  | Spasms                                 | 16                       |
| <b>Total</b>                     |  | <b>114</b>               |

### 3.3.7.3.6 Other features at presentation

**Inter-current illness, infection or vaccination (n=41)** occurring in the preceding seven days was considered significant. The following intercurrent illnesses were documented:

- Infections (n=32) which included upper respiratory tract infections (URTI) (n=13), gastroenteritis (n= 7), fever and no other symptoms (n=6), fever and rash (n= 2), fever and headache (n=2), and fever and other nonspecific symptoms (n=2). 21 out of these 32 patients had a documented temperature of 37.8 C° or higher (axillary) at the time of the presenting seizure. 37.8 C is the temperature used to define febrile seizures (Berg 1992 Feb seizures)).
- Other intercurrent illnesses (n=3) included asthma exacerbation (n=1), cardiac surgery (n=1), and elevated blood pressure (n=1).
- History of vaccination in the preceding week (n=5).
- Infliximab infusion in the preceding week (n=1).

**Other neurological and neuro-psychiatric features (n=48)** were recorded when they occurred within the first week of seizure presentation, including one or more of the following features:

- **Encephalopathy (n=33)** was defined by the presence of lethargy, drowsiness, reduced responsiveness or altered level of consciousness (reported by the carer or clinician) that lasted more than 24 hours and was not attributed to the post ictal state (Glaser 2003).
- **Behavioural or psychiatric alteration (n=27)** was defined by the presence of agitation, emotional lability, irritability, or other changes from normal behaviour as reported by the carer or clinicians. Behavioural alteration occurred mostly in patients who also had encephalopathy (above).
- **Motor impairment (n=17)** of new onset and not attributed to drug side effects included ataxia, limb weakness (including Todd's paresis), swallowing difficulties, slurred speech or hypotonia reported in association with seizure onset.



- **Cognitive alteration (n=12)** was difficult to ascertain particularly for young children and infants and was defined by the presence of memory or intellectual concerns reported in the clinical record, from parental concerns, clinical assessment or standardised testing when performed.
- **Movement disorder (n=5)** was reported occasionally including tremor in three, dystonia in one and dyskinesia in three (more than one movement disorder reported in some individuals).

#### **3.3.7.4 Investigations of the cohort**

The investigation was requested by the attending clinician as clinically indicated for the individual case. No further investigations were performed for the purpose of this study apart from neuronal antibody assays. The following investigations were performed for the 114 patients:

##### **3.3.7.4.1 EEG/Video EEG**

An EEG was done in 113 out of the 114 patients and was abnormal in 86 out of the 113 done (76.1%). A degree of slowing (focal, diffuse, continuous or intermittent) was found in 62 patients (54.9%). Epileptic activity was found in 58 patients (51.3%). Clinical or electrical seizures were recorded in 27 patients (23.9%). In 22 patients the EEG findings contributed significantly to the diagnosis including: hypsarrhythmia, burst suppression, epileptic encephalopathy, abnormalities supportive of electroclinical syndromes (West syndrome, Childhood absence epilepsy, Benign epilepsy with centrotemporal spikes) and abnormalities suggestive of focal epilepsy due to a possible structural cause.

##### **3.3.7.4.2 Brain MRI**

MRI scan of the brain was done in 105 out of the 114 patients (92.1%) and showed an abnormality in 52 patients out of the 105 done (49.5%). MRI was diagnostic or suggestive of a cause of the epilepsy in 30 patients (28.6%), including malformation of cortical development (n=12), encephalitis (n=5), encephalomalacia (n=4), neurocutaneous syndrome (n=3), occipital gliosis suggestive of neonatal hypoglycemia (n=2), metabolic disorders (n=2, Mitochondrial encephalomyopathy, lactic acidosis,

and stroke-like episodes- MELAS and propionic academia), Rasmussen encephalitis (n=1) and posterior reversible encephalopathy syndrome (PRES) (n=1).

#### **3.3.7.4.3 CSF**

CSF analysis was performed in 66 out of the 114 patients (57.9%) and showed the following abnormalities: pleocytosis (defined as white cells in excess of  $5/\text{mm}^3$ ) (n=7), elevated neopterin suggestive of inflammation ( $>30 \text{ nmol/L}$ ) (n=22, 55 tested), abnormal oligoclonal bands (n=2, 41 tested) (with mirrored serum bands in one and intrathecal bands in one). CSF viral PCRs were performed as follows: Herpes Simplex Virus (HSV) (n=27, all negative), enterovirus (n=26, two positive), Varicella Zoster Virus (VZV) (n=4, all negative), Human Herpes Virus 6 (HHV6) (n=3, one positive) and Cytomegalovirus (CMV) (n=1, negative).

#### **3.3.7.4.4 Other Investigations**

Other investigations were performed including metabolic, imaging, genetic, infectious and immunologic investigations were recorded when performed. In six patients these investigations helped establish a diagnosis, including the metabolic disorders congenital glycosylation disorder (CGD) (n=2) and MELAS (n=1). In addition, genetic abnormalities were found in three including genetic mutations or abnormalities as follows: PCDH19 (n=1), SCN1A (n=1) and deletion of 22q11.2 (n=1).

#### **3.3.7.5 Treatment**

This study was not designed to study acute or long term treatment of seizures. Treatment received was recorded by listing the antiepileptic medications used (acutely or on long term). The use of steroids and/ or IVIG was recorded as well as the response of seizures.

Forty-six patients received acute seizure treatment including a single medication or combination of the following: midazolam (buccal/nasal or intravenous) (n=30), phenytoin (n=27), phenobarbitone (n=6), clobazam (oral) (n=4), pyridoxine (intravenous) (n=3), levetiracetam ((intravenous) (n=1) and thiopentone (n=1).

Twenty-nine patients received no long term antiepileptic treatment, defined as more than seven days. 85 patients received long term antiepileptic drugs (AEDs) including one AED (n=38), two AEDs (n=18) and three or more AEDs (n=29) (as single agents or in combination) during the period of follow up.

Twenty-three out of 114 received variable regimens of steroids, including steroids alone (oral prednisolone and intravenous methyl prednisolone) in 18 and steroids and intravenous immunoglobulins (IVIG) in five. A positive response to steroids given alone was reported in 14 patients, including West syndrome (n=6), epilepsy of unknown cause (n=4), Lennox Gastaut syndrome (n=2), epilepsy attributed to malformation of cortical development (n=1) and epilepsy attributed to perinatal insult (encephalomalacia, n=1). A positive response to steroids given in combination with IVIG was reported in 2 patients including Rasmussen encephalitis (n=1) and basal ganglia encephalitis (n=1).

### **3.3.7.6 Outcome**

The outcome was studied by reviewing the last follow up recorded on the electronic recording system, either through attending outpatient clinic or hospital emergency, hospital admission or through follow up phone calls made by the caring teams. 10 patients (8.8 %) had no follow up information available. For the remaining 104 patients, the mean length of follow up (from seizure onset) was 11.7 months (median 11 months, range 1-36 months).

The following outcome measures were used:

#### **3.3.7.6.1 Epilepsy outcome**

The seizure or epilepsy outcome was recorded for the 104 patients with natural follow up as follows:

1. **Isolated event (n=20)** where the patient had no further seizures during follow-up. For example a patient with encephalitis who had seizures on presentation with the encephalitis illness (single or multiple) but had no further seizures on follow up (without being on long term antiepileptic medications) was considered to have an isolated event.

2. **Ongoing epilepsy (n=84)** was recorded if the patient had further seizures beyond the acute presenting seizures. This includes those who have achieved seizure control on one or more antiepileptic medications. Of the 84 patients with ongoing epilepsy, 23 (27.3%) had drug resistant (refractory epilepsy) which was defined by as per ILAE special report 2010 as “failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drugs (as monotherapy or in combination) to achieve sustained seizure freedom” (Kwan et al., 2010).

### 3.3.7.6.2 Other outcome measures

A new deficit (other than epilepsy) was present in 32 patients, this included developmental delay/ learning difficulties/ cognitive impairment, behavioural/ psychiatric impairment or motor impairment that were not present prior to the onset of seizures.

The following new deficits were recorded at follow up:

- a. **Development delay/ cognitive impairment (n=24)** as reported by family or treating clinician was defined as failure to achieve age appropriate developmental milestones (in young children) or learning difficulties and concerns regarding cognitive function (in older children), or when these were evident on standardized testing.
- b. **Behavioral/ psychiatric disturbance (n=18)** were recorded such as anxiety, emotional lability, depression, aggressive behavior and attention deficit hyperactivity disorder.
- c. **Motor or focal deficits (n=13)** were recorded such as hypotonia, spasticity, ataxia, weakness, sensory deficits, visual impairment and slurred speech.

### 3.3.8 ILAE classification of epilepsy syndromes and epilepsy aetiology

Classifications of patients' seizures, epilepsy type, electroclinical syndrome and aetiology was performed by the primary investigator (JS) and cross checked by an associate supervisor who is a Paediatric Neurologist and Epileptologist (DG). Both the primary investigator and the associate

supervisor were blinded to the results of the antibody testing at the time of performing the classification.

Classification of patients into an epilepsy syndrome was made by reviewing the child's age, neurological and developmental state, seizure semiology (at onset and on follow up), EEG, imaging and other relevant investigations findings.

The latest proposed ILAE classification was used for this purpose (Berg et al., 2010). The major difference of the latest ILAE 2010 report to previous reports was the replacement of the previous terms "idiopathic" with "genetic", "symptomatic" with "structural-metabolic", and "cryptogenic" with "unknown" (see Chapter 1).

The following categories were used for the patients' classification:

### ***3.3.8.1 Electroclinical Syndromes (n=33)***

Patients who had an electroclinical syndrome "as described in ILAE classifications (Engel, 2001, Berg et al., 2010)" were classified as such irrespective of the aetiology of their epilepsy and its syndrome. For example patients with epileptic spasms and hypsarrythmic EEG were classified as West syndrome despite some of these patients having a known structural or metabolic cause. The syndromes were described according to the patient age as per ILAE classifications.

In addition to the electroclinical syndromes described in the latest ILAE classification we found that some of our patients had generalised seizures with no underlying structural brain lesion or other neurological signs or symptoms. These patients do fit into the old classification "idiopathic generalised epilepsy" which is presumed to be genetic (Engel, 2001). The term "idiopathic" is not recommended by the new ILAE classification by Berg et al; however we used it to classify these patients as we did find it useful in this setting- these patients were classified as "idiopathic generalised epilepsy not otherwise specified", see Table 3-3.

### ***3.3.8.2 Epilepsy attributed to structural-metabolic causes (n=33)***

Patients who did not fit into any electroclinical syndrome were classified (as per the ILAE 2010 recommendation) on the basis of presence of metabolic diseases or structural abnormalities of the brain such as malformation of cortical development, neurocutaneous syndromes, infections and other structural or metabolic causes, see Table 3-3 and 3-4.

### ***3.3.8.3 Epilepsy of unknown cause (n=30)***

The term “epilepsy of unknown cause” was used to classify other patients who do not fit into an electroclinical syndrome and who have no known structural, metabolic or genetic cause for their epilepsy. These patients were further sub-classified according to whether the seizures were focal, generalised or spasms. In some patients it was difficult to determine whether their epilepsy was focal or generalised as they had both focal and general seizures and EEG was normal or showed both focal and generalised abnormalities, and the term “undetermined” was used in these cases as per the previous ILAE commission report (Roger et al., 1989).

### ***3.3.8.4 Seizures that are traditionally not diagnosed as a form of epilepsy (n=18)***

Patients with epileptic seizures that are not traditionally diagnosed as a form of epilepsy were kept in a separate group. This included the following groups:

1. Febrile seizures (n=7) were defined as seizures occurring in previously normal children aged 6 months-5 years, who had a documented temperature of 37.8 C° (axillary) or 38.5 C° (rectal) or higher at the time of the presenting seizure (Berg et al., 1992) in the absence of CNS infection or inflammation or another cause for the seizures. Upon follow-up these children either developed no further seizures or had further febrile seizures and developed no neurological or developmental abnormalities. Children who presented with febrile seizures but developed afebrile or unprovoked seizures later on (particularly if they had family history of epilepsy) were classified as febrile seizure plus (FS+). Children who continued to have

febrile seizures with significant neuro-developmental deterioration were classified as Dravet Syndrome, if the other clinical and age characteristics were consistent with Dravet syndrome.

2. Acute symptomatic (provoked) seizure (n=9) was the term used for children who had systemic insults at the time of presentation, including systemic infections (without fulfilling the above criteria for febrile seizures), or other acute CNS insult (Beghi et al., 2010, Beleza, 2012). We did not include patients with encephalitis here as some patients with encephalitis developed long term epilepsy. Instead we classified the encephalitis patients in the subgroup attributed to structural metabolic causes (see Table 3-3). Patients who had encephalitis are described in more details at the end of this section.
3. Unprovoked seizure (n=2) was the term used to describe a single seizure occurring in the absence of any identified systemic or CNS insult. Patients with unprovoked seizures are thought to have a different risk of seizure recurrence (Beghi et al., 2010).

The ILAE classification and electroclinical syndromes of the 114 patients are presented in Table 3-3.

*Table 3-3 Epilepsy ILAE classification/electro-clinical syndromes and others*

*(as per the recommended structure by Berg et al 2010)*

| <b>ILAE classification/ electro-clinical syndrome</b>  |   | <b>Patients (number)</b> |
|--|---|--------------------------|
| <b><i>Electroclinical syndrome (n=33)<sup>a</sup></i></b>  |   |                          |
| Infancy  |   |                          |
|  | West syndrome   | 9 <sup>b</sup>           |
|  | Benign infantile epilepsy   | 3                        |
|  | Dravet Syndrome   | 3                        |
|  | Myoclonic epilepsy in infancy (MEI)   | 1                        |
| Childhood  |   |                          |
|  | Lennox Gastaut Syndrome (LGS)   | 4 <sup>c</sup>           |
|  | Febrile seizures plus (FS+)   | 2                        |
|  | Panayiotopoulos syndrome  | 2                        |
|  | Epilepsy with myoclonic atonic seizures                                       | 2                        |
|  | Benign epilepsy with centrotemporal spikes (BECTS)                            | 1                        |
|  | Childhood absence epilepsy (CAE)  | 1                        |
|  | Late onset Childhood occipital epilepsy (Gastaut type)                        | 1                        |
| Adolescence  |   |                          |
|  | Autosomal dominant epilepsy with auditory features (ADEAF)                    | 1                        |
| Idiopathic generalised epilepsy (not otherwise specified) <sup>d</sup>   |   | 3                        |
| <b><i>Epilepsy attributed to structural-metabolic causes (n= 33)</i></b>   |   |                          |
|  | Infections/inflammation   |                          |
|  | Encephalitis  | 11 <sup>e</sup>          |
|  | Rasmussen Syndrome  | 1                        |
|  | Malformation of cortical development (MCD)                                    | 10                       |
|  | Perinatal insults   | 5                        |
|  | Metabolic   | 3 <sup>f</sup>           |
|  | Neurocutaneous syndrome   | 3                        |
| <b><i>Epilepsies of unknown cause (n=30)</i></b>   |   |                          |
|  | Focal   | 16                       |
|  | Generalised   | 7                        |
|  | Spasms  | 4                        |
|  | Undetermined  | 3                        |
| <b><i>Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy (n=18)</i></b> |   |                          |
|  | Febrile seizures  | 7                        |
|  | Acute symptomatic (provoked seizures)   |                          |
|  | Infection mediated  | 7 <sup>g</sup>           |
|  | Posterior reversible encephalopathy syndrome (PRES) secondary to hypertension | 1                        |
|  | Asthma  | 1                        |
|  | Single unprovoked seizure   | 2                        |
| <b>Total</b>   |   | <b>114</b>               |



<sup>a</sup> "The arrangement of electroclinical syndromes does not reflect etiology" (Berg 2010)

<sup>b</sup> 3 genetic, 2 metabolic (Congenital glycosylation disorder), 1 structural (perinatal insult), 3 unknown

<sup>c</sup> 2 structural (malformation of cortical development, holoprosencephaly), 2 unknown

<sup>d</sup> "Idiopathic generalised epilepsy" was used here though not part of the new classification, as the investigators found it useful for these patients

<sup>e</sup> Encephalitis patients include the following: five with potential infectious aetiology, one with dopamine receptor 2 encephalitis, and five not otherwise specified.

<sup>f</sup> MELAS, Propionic acidemia, Ornithine transcarbamylase deficiency

<sup>g</sup> These patients had a systemic infection that was presumed to have provoked the seizure, but no evidence of CNS infection or inflammation, and either had no fever or were outside the age definition for febrile seizures. The precipitating infections were gastroenteritis (n=3), URTI (n=2), H1N1 influenza (n=1), fever alone (n=1).

### **3.3.8.5 ILAE aetiology classification**

A second level of classification was performed according to the aetiology of epilepsy regardless of the epilepsy syndrome classification- this was performed by the primary investigator (JS) blinded to the results on antibody studies.

96 out of the 114 patients had epilepsy and were classified according to the aetiology of their epilepsy irrespective of their epilepsy syndrome into structural-metabolic (n=36), genetic (or presumed genetic) (n=25), and unknown (n=35), see Table 3-4. The remaining 18 patients had conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy and were not included in this aetiology classification, although they are included in Table 3-4 for completeness. Patients with these conditions are generally thought to have a genetic predisposition for their seizures.

*Table 3-4 Epilepsy aetiology classification for patient cohort as per Berg 2010*

| <b>Aetiology</b>   |   | <b>Patients<br/>(number)</b> |
|--|---|------------------------------|
| <b><i>Structural- metabolic (n=36)</i></b>   |   |                              |
| Infection/inflammation (n=12)  |   |                              |
|  | Encephalitis  | 11                           |
|  | Rasmussen Syndrome  | 1                            |
| Malformation of cortical development (MCD) (n=12)  |   |                              |
|  | Focal cortical dysplasia (FCD)  | 5*                           |
|  | Polymicrogyria  | 3                            |
|  | Subcortical band heterotopias   | 1                            |
|  | Periventricular nodular heterotopia   | 1                            |
|  | Pachygyria  | 1                            |
|  | Holoprosencephaly   | 1                            |
| Metabolic (n=5)  |   |                              |
|  | Congenital Disorders of Glycosylation   | 2                            |
|  | Ornithine transcarbamylase deficiency   | 1                            |
|  | Propionic acidemia  | 1                            |
|  | MELAS   | 1                            |
| Perinatal insult (n=6)   |   |                              |
|  | Occipital gliosis secondary to neonatal hypoglycaemia                         | 2                            |
|  | Cerebral atrophy secondary to old ischaemic infarctions                       | 2                            |
|  | Encephalomalacia secondary to intraventricular hemorrhage (prematurity)       | 2                            |
| Neurocutaneous syndrome (n=3)  |   |                              |
|  | Tuberous sclerosis  | 1                            |
|  | Neurofibromatosis type 1  | 1                            |
|  | Sturge Weber Syndrome   | 1                            |
| <b><i>Genetic (n=25)</i></b>   |   |                              |
| Presumed genetic (n=20)  |   | 20                           |
| Chromosomal (n=3)  |   |                              |
|  | Trisomy 21  | 2                            |
|  | Tetrasomy 15  | 1                            |
| Monogenic (n=2)  |   |                              |
|  | SCN1A   | 1                            |
|  | PCDH19  | 1                            |
| <b><i>Unknown (n=35)</i></b>   |   |                              |
| <b><i>Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy (n=18)</i></b> |   |                              |
| Febrile seizures (n=7)   |   | 7                            |
| Acute symptomatic seizures (provoked) (n=9)  |   |                              |
|  | Systemic infection (n=7)  |                              |
|  | Gastroenteritis   | 3                            |
|  | URTI  | 2                            |
|  | H1N1 influenza  | 1                            |
|  | Fever   | 1                            |
|  | Posterior reversible encephalopathy syndrome (PRES) secondary to hypertension | 1                            |
|  | Asthma  | 1                            |
| Unprovoked seizures (n=2)  |   | 2                            |
| <b><i>Total</i></b>  |   | <b><i>114</i></b>            |

\*one patient had FCD presumed secondary to genetic abnormality (14q21deletion), we elected to keep in structural metabolic group rather than genetic.

### **Encephalitis (n=11)**

Eleven patients had encephalitis as per Tables 3-3 and 3-4. All patients fulfilled criteria of encephalitis as per Granerod (Granerod et al., 2010). As this group was heterogeneous in aetiology we describe these patients in more details here. A potential infectious cause was found in five patients including: a virus isolated from CSF (n=2) (enterovirus and human herpes simplex virus 6), positive serology (n=2) (mycoplasma pneumoniae and adenovirus) and positive nasopharyngeal aspirate (n=1) (influenza B virus). One patient had basal ganglia encephalitis and was dopamine receptor 2 positive (Dale et al., 2012). Five patients had no aetiology found. Two patients had clinical and radiographic features of limbic encephalitis (one with positive human herpes simplex virus 6 and one with no aetiology found).

### **3.3.9 Controls**

The control group used in this study is different to that used in Chapter 2. This control group consisted of hospital patients who had serum collected as part of their routine investigations (for infectious or immunological tests) during the year 2007. This group included 65 control patients (27 females, mean age 9 years, median 9.14 years, range: 1-16 years). The underlying medical problems were immunologic/ inflammatory or allergic (n=28), haematologic/oncologic (n=20) or other medical condition (n=14), see Table 3-5.

The 65 control patients were tested at the same time as the patients for the following antibodies: LGI1, CASPR2, contactin-2, NMDAR, GAD, Gly R (n=65) and VGKC-complex (n=62, due to run out of samples for three controls).

*Table 3-5 Classification of control group (n=65)*

| <b>Group</b>                                      | <b>Subgroup</b>   | <b>Patients (number)</b> |
|---|---|--------------------------|
| <b><i>Immune/allergic/inflammatory (n=28)</i></b> |   |                          |
|   | Type 1 diabetes mellitus                                  | 8                        |
|   | Asthma/food allergies                                     | 5                        |
|   | Crohn disease/ulcerative colitis                          | 4                        |
|   | Familial periodic fever                                   | 3                        |
|   | Systemic lupus erythematosus                              | 3                        |
|   | Immunodeficiency  | 2                        |
|   | Arthritis (post streptococcal)                            | 1                        |
|   | Uveitis   | 1                        |
|   | Idiopathic thrombocytopaenia purpura                      | 1                        |
| <b><i>Hematologic/oncologic (n=21)</i></b>        |   |                          |
|   | Acute leukemia  | 9                        |
|   | Neuroblastoma   | 3                        |
|   | Fanconi Anaemia   | 2                        |
|   | Wiscott Aldrich Syndrome                                  | 2                        |
|   | Familial platelet disorder                                | 2                        |
|   | Hemophagocytic lymphohistiocytosis                        | 1                        |
|   | Osteosarcoma  | 1                        |
|   | Bone marrow transplant (for Adrenal Leukodystrophy)       | 1                        |
| <b><i>Other medical (n=16)</i></b>                |   |                          |
|   | Respiratory (Cystic fibrosis, bronchopulmonary dysplasia) | 3                        |
|   | Nutritional and growth (poor weight gain, growth delay)   | 3                        |
|   | Endocrine (Thyroid dysgenesis, multiple endocrinopathies) | 2                        |
|   | Recurrent infections (no immunodeficiency)                | 2                        |
|   | Cardiac (cardiomyopathy)                                  | 1                        |
|   | Gastrointestinal (chronic diarrhea)                       | 1                        |
|   | Acute infection   | 1                        |
|   | Post viral fatigue and positive ANA                       | 1                        |
|   | Tic disorder, Tourette syndrome                           | 1                        |
|   | Prader Willi syndrome                                     | 1                        |
| <b><i>Total</i></b>                               |   | <b>65</b>                |

### 3.3.10 Statistics

Fisher exact test was used to calculate the P value and analyse 2x2 contingency tables for categorical data, while Mann-Whitney test was used to compare continuous data.

## **3.4 Results of neuronal antibodies**

### **3.4.1 Results of patients (n=114)**

11 out of 114 (9.65%) patients with seizures were positive for one or more of the tested antibodies, VGKC-complex (n=6), NMDAR (n=4) and CASPR2 (n=3). Two patients were double positive for VGKC-complex and NMDAR Abs. None of the patients was positive for LGI1 or GAD antibodies.

#### ***3.4.1.1 Clinical features of positive patients (n=11)***

The 11 positive patients (4 females) had a mean age of 4.4 years (median 3.4, age range 0.5-10 years). The positive cases are presented in Table 3-6.

All 11 patients had new onset seizures; however on further review one patient (case 11) had a possible febrile seizure at the age of 23 months. Nine patients had no preexisting neurologic abnormality and two had pre-existing developmental delay, which was severe in one. These two patients with preceding neurological abnormalities had underlying neurologic conditions including microform holoprosencephaly in one, and dysmorphic features and possible undiagnosed syndrome in the other. Four patients had medical conditions including Diabetes Insipidus (n=1), Crohn's disease (n=1), short stature (n=1) and hip dysplasia (n=1). There was family history of epilepsy in one patient and of febrile seizures in a further patient.

The presenting seizure was focal in six, generalised in 4 and focal with secondary generalisation in one. The presenting seizure was associated with fever in one patient (case 6). One patient had status epilepticus at presentation of 45 minutes duration (case 8). Early seizure recurrence in the first 48 hours of presentation occurred in seven out of the 11 patients.

*Table 3-6 Demographic, clinical, electrographic and imaging features of positive patients (n=11)*

| <b>No.</b> | <b>Age and sex</b> | <b>Seizure type at onset/early seizure recurrence</b> | <b>Associated features</b>                    | <b>EEG *</b>  | <b>MRI</b>                                     | <b>Seizure type on follow up</b>          | <b>Number of AED on follow up (months)</b> | <b>ILAE classification</b>                      | <b>Ab positivity \$</b>           |
|------------|--------------------|---|---|---|--|---|--|---|-----------------------------------|
| <b>1</b>   | 2.5M               | Focal dyscognitive/+                                  | Nil   | Epileptic: left fronto-temporal<br>Seizure: left temporal | Normal   | Focal dyscognitive, atypical absence      | 1 (11)                                     | Epilepsy of unknown cause (focal-temporal lobe) | <b>VGKC (293)</b>                 |
| <b>2</b>   | 4.9M               | Focal versive/-                                       | Nil   | Epileptic: generalised with bifrontal lead                | Normal   | Generalised tonic clonic                  | 1 (6)                                      | Epilepsy of unknown cause (undetermined)        | <b>VGKC (193)</b>                 |
| <b>3</b>   | 3.4M               | Generalised tonic clonic/+                            | Nil   | Slowing: right posterior                                  | Normal   | Generalised tonic clonic, atonic          | 2 (6)                                      | Epilepsy of unknown cause (generalised)         | <b>VGKC (182)</b>                 |
| <b>4</b>   | 1.5F               | Focal dyscognitive/+                                  | Motor deficit (ataxia)                        | Normal  | Normal   | Focal dyscognitive, atypical absence      | 1 (17)                                     | Epilepsy of unknown cause (focal)               | <b>VGKC (133)</b>                 |
| <b>5</b>   | 0.5F               | Focal tonic with secondary generalisation/+           | Intercurrent infection                        | Slowing: right temporal<br>Epileptic: central             | Normal   | Focal tonic with secondary generalisation | 1 (11)                                     | Epilepsy of unknown cause (focal)               | <b>CASPR2 (2)</b>                 |
| <b>6</b>   | 7M                 | Focal tonic clonic/-                                  | Intercurrent infection<br>Fever               | Not done  | Not done                                       | No follow up                              | -  | Acute symptomatic (provoked) seizure            | <b>CASPR2 (1)</b>                 |
| <b>7</b>   | 10M                | Focal myoclonic/+                                     | Encephalopathy<br>Motor deficit (hemiparesis) | Slowing: generalised<br>Epileptic: left parietal.         | Left parietal hyperintensity                   | Focal                                     | 2 (13)                                     | Epilepsy attributed to metabolic cause (MELAS)  | <b>CASPR2 (2.5)</b>               |
| <b>8</b>   | 4.5M               | Focal dyscognitive/-                                  | Nil   | Slowing: right occipital.                                 | Normal   | No follow up                              | -  | Epilepsy of unknown cause (focal-occipital)     | <b>NMDAR (1.5)</b>                |
| <b>9</b>   | 3M                 | Generalised Myoclonic /+                              | Encephalopathy<br>Behavioural alteration      | Slowing: generalised<br>Epileptic: right frontal.         | Corpus callosal dygenesis, frontal heterotopia | Mixed (myoclonic, tonic, atonic)          | 4 (12)                                     | Lennox- Gastaut syndrome                        | <b>NMDAR (1.5)</b>                |
| <b>10</b>  | 8F                 | Generalised tonic/-                                   | Preceding infliximab infusion                 | High voltage generalised spike and slow wave              | Generous ventricles                            | Tonic, myoclonic                          | 1 (10)                                     | Epilepsy of unknown cause (generalised)         | <b>VGKC (368)<br/>NMDAR (1.5)</b> |
| <b>11</b>  | 3F                 | Generalised tonic clonic/+                            | Intercurrent infection                        | Normal  | Asymmetric hippocampi                          | Generalised tonic clonic                  | 2 (7)                                      | Febrile seizure plus (FS+)                      | <b>VGKC (233)<br/>NMDAR (1.5)</b> |

\* EEG abnormal findings described in the following order when present: slowing and location, epileptic activity and location, seizure and onset. + present, - Absent

\$ VGKC Abs >100pM are considered positive, CASPR2 score of more than 1 and NMDAR score of more than 1.5 are considered positive (0 no binding- 4 strong binding)

The associated features on presentation included: intercurrent infection (n=3), encephalopathy (n=2), motor impairment (n=2) (ataxia and right hemiparesis), behavioural alteration (n=1) and fever (n=1). One patient had infliximab infusion one day prior to seizures onset for the treatment of her Crohn's disease (case 10).

All 11 patients were admitted to hospital with a mean duration of hospital stay of 3.72 days (median 3 days, range 2-11 days). None of the 11 patients required admission to the intensive care unit.

#### ***3.4.1.2 Investigations of positive patients***

An EEG was done in 10 out of the 11 positive patients and was abnormal in eight. Slowing was present in five cases (focal in four and generalised in one) and epileptic discharges were present in six cases (focal in four and generalised in two). A tonic seizure with left temporal onset was recorded in one patient (case 1).

MRI brain was done in 10 out of 11 patients and was abnormal in four. One had a known preexisting structural abnormality. The abnormalities found include generous CSF spaces (n=1), asymmetry in hippocampi without abnormal signal (n=1), corpus callosum dysgenesis and periventricular heterotopia (n=1) and focal cortical hyperintensity suggestive of MELAS (n=1). MRI was diagnostic in two cases (MELAS and heterotopia).

CSF analysis was performed in four cases. The following CSF indices were negative or normal in tested patients: cell count (n=4), protein (n=3), oligoclonal bands (n=3), culture (n=4) and PCR for HSV (n=1) and enterovirus (n=1). CSF neopterin was elevated in one out of three tested at 68 nmol/L (normal <30) (case 1).

Other positive investigations performed for the patients include low serum sodium 128 (case 6), elevated serum lactate (3.6 mmol/L, normal <2.0) and positive m.3243A>G mutation suggestive of MELAS (case 7), parainfluenza virus detected in nasopharyngeal aspirate (case 5), and duplication of chromosome 7 found on array-comparative genomic hybridization (a-



CGH) (case 1). This duplication involved a segment of 0.24 Mb in bands q22.3 to q31.1 and contained 7 known genes, two of which are associated with OMIM-listed disease (SLC26A4 & SLC26A3) but none of these genes have been associated with seizures. The same duplication was found in his mother who is healthy. It was concluded that the duplication is a familial variant and of no clinical significance.

#### ***3.4.1.3 Treatment of positive patients***

Four patients out of the 11 positive patients received acute treatment for their first seizure including phenytoin (n=3) and midazolam (n=1). Nine patients received long term antiepileptic drugs (AED). None of the 11 positive patients received steroids or IVIG at any stage.

#### ***3.4.1.4 Outcome of positive patients***

Two cases were lost to follow up (cases 6 and 8). The mean length of follow up for the remaining nine cases was 10.33 months (median 11 months, range 6-11 months).

All of the nine patients with follow up had ongoing epilepsy and one had drug resistant epilepsy (case 9). At the time of follow up five patients were on one AED, three were on two AEDs and one was on four AEDs. Two patients had a new neurologic or developmental deficit (other than epilepsy) including speech impairment and hyperactivity (case 3), and motor deficit and behavioural alteration (case 5).

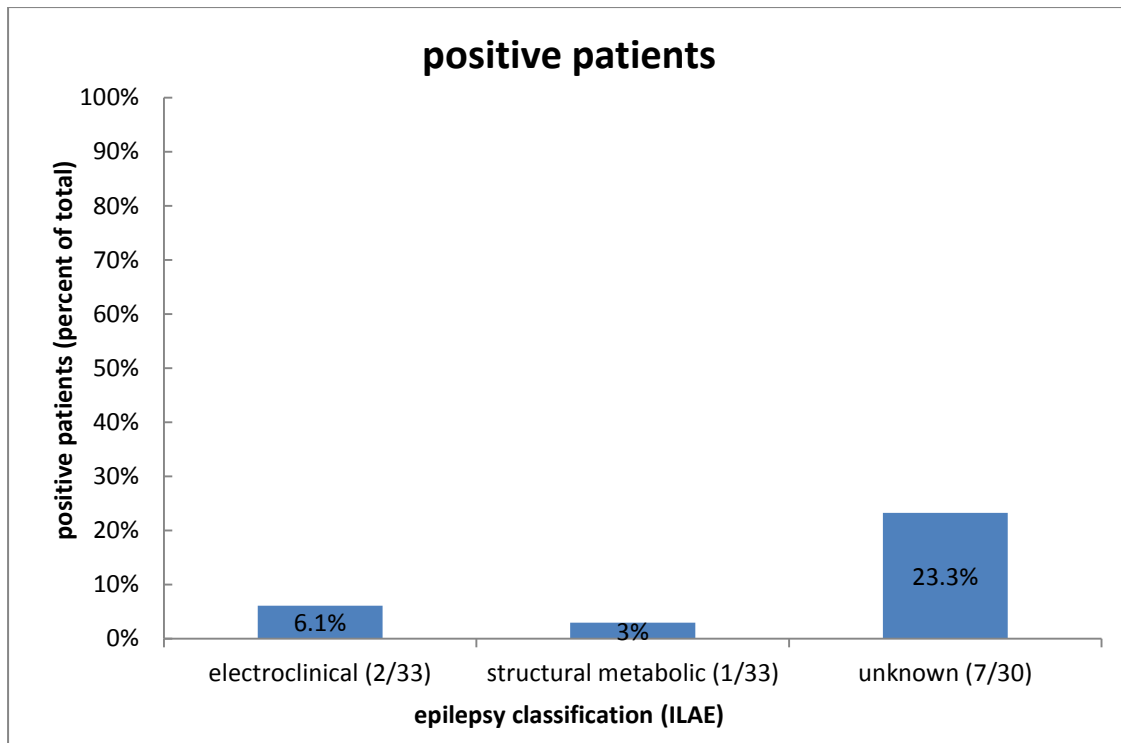
#### ***3.4.1.5 ILAE Classifications of positive patients***

The 11 Ab positive patients had the following ILAE epilepsy classifications: electroclinical syndrome (n=2), epilepsy attributed to structural metabolic causes (n=1) and epilepsy of unknown cause (n=7). The 11th patient had an acute symptomatic seizure (which is not considered a form of epilepsy).

The positive patients in different categories of ILAE classifications are presented in Figure 3.3 as a percentage of the total patients in each group. Only 2/ 33 patients with an electroclinical syndrome (6.1%) had positive antibodies (cases 9 and 11), which was not significantly different to the positive Abs in the control group, see below (3/65, 4.6%). Case 9 had Lennox Gastaut Syndrome (LGS) and antibodies to NMDAR, and case 11 had febrile seizures plus with antibodies to both the NMDAR and VGKC-complex.

Only 1/ 33 patients with epilepsy attributed to a structural-metabolic cause (3%) had positive antibodies (case 7) which was not significantly different to the controls. Case 7 had MELAS and was positive for CASPR2 antibodies.

In comparison, 7/30 of patients with epilepsy of unknown cause (23.3%) had positive antibodies, which was significantly different to the controls (4.6%,  $p=0.01$ , Fisher's exact). Of these seven antibody positive patients with epilepsy of unknown cause, four had VGKC-antibodies only (cases 1-4), three of whom presented with focal seizures, and three had early seizure recurrence (cases 1, 3 and 4). Case 5 had CASPR2 antibodies, presented with focal tonic/clonic seizures and had intercurrent infections. Case 8 was positive for NMDAR-Abs and had focal dyscognitive seizures and focal status epilepticus on presentation. Case 10 was double positive for NMDAR and VGKC-complex and had a number of co-morbidities including developmental delay and treatment resistant Crohn's disease; she presented with seizures following an infliximab infusion, a monoclonal antibody used to treat autoimmune diseases which has been previously associated with seizures (Brigo et al., 2011).



*Figure 3.3 ILAE classifications of patients positive for one or more antibodies (expressed as percentage of total).*

#### **3.4.1.6 Comparison between positive and negative patients with seizures**

There was no difference in the demographic features of positive and negative patients. There were seven males out of the 11 positive patients (63.63%) compared to 49 males out of the 103 negative patients (47.57%) (  $p=0.36$ , Fisher's exact test). The age mean was 4.39 years for both positive and negative patients (  $p$  value 0.42, Mann-Whitney test). The median age for positive patients was 3.4 years and that for the negative patients was 2 years. The mean for serum sample collection time (from seizure onset) was 17.55 days for the positive patients and 40 days for the negative patients (  $P=0.16$ , Mann-Whitney test). The median time for serum sample timing was 4 days for positive patients and 20 days for negative patients.

There was no significant difference between the positive and negative seizure patients in clinical characteristics including past medical history, presenting seizure type, associated features or outcome, see Table 3-7.

There was a statistically significant difference in the classification of epilepsy between positive and negative patients. The proportion of antibody positive patients in the epilepsy of unknown cause category (7/11; 63%) was significantly higher compared to the remaining antibody negative patients (23/103; 22 %;  $p=0.007$ , Fisher's exact test). Moreover, in the antibody positive patient group, 4 out of 11 had *focal* epilepsy of unknown cause (36.4%) compared with only 12 of the 103 antibody-negative patients (11.7%;  $p=0.047$ , not corrected for multiple comparisons), see Table 3-7.

*Table 3-7 Comparison of features of positive and negative cases by ILAE Classification and clinical characteristics*

| Characteristic  | Presence of characteristic in Ab positive patients (%) n=11 | Presence of characteristic in Ab negative patients (%) n=103 | P value      |
|---|---|--|--------------|
| <b>Past medical history</b>                               |   |  |              |
| Previously normal (n=78)                                  | 9 (81.81)   | 69 (66.99)   | 0.50         |
| <b>First Seizure</b>                                      |   |  |              |
| Focal   | 7 (63.63)   | 49 (47.57)   | 0.36         |
| Generalised   | 4 (36.36)   | 38 (36.89)   | 1.00         |
| Status epilepticus  | 1 (9.09)  | 20 (19.41)   | 0.69         |
| Early seizure recurrence                                  | 7 (63.63)   | 70 (67.96)   | 1.00         |
| <b>Hospitalisation (n=101)</b>                            |   |  |              |
| Intensive care (n=18)                                     | 0 (0.0)   | 18 (17.48)   | 0.21         |
| <b>Associated features</b>                                |   |  |              |
| Fever   | 1 (9.09)  | 20 (19.41)   | 0.69         |
| Infection   | 3 (27.27)   | 28 (27.18)   | 1.00         |
| Encephalopathy  | 2 (18.18)   | 31 (30.08)   | 0.51         |
| Movement disorder   | 0 (0.0)   | 5/ (4.85)  | 1.00         |
| Behavioural abnormality                                   | 1 (9.09)  | 26 (25.24)   | 0.45         |
| Cognitive abnormality                                     | 0 (0.0)   | 12 (11.65)   | 0.60         |
| Motor deficit   | 2 (18.18)   | 15 (14.56)   | 0.67         |
| <b>Outcome</b>  |   |  |              |
| Ongoing epilepsy  | 9 (81.81)   | 75 (72.82)   | 0.72         |
| New deficit   | 2 (18.18)   | 30 (29.13)   | 0.73         |
| Developmental delay                                       | 0 (0.0)   | 16 (15.53)   | 0.36         |
| Behavioural /psych impairment                             | 2 (18.18)   | 16 (15.53)   | 0.68         |
| Cognitive impairment                                      | 0 (0.0)   | 17 (16.50)   | 0.36         |
| <b>ILAE Classification</b>                                |   |  |              |
| Electroclinical syndrome (n=33)                           | 2 (18.18)   | 31 (30.1)  | 0.51         |
| Epilepsy attributed to structural-metabolic causes (n=33) | 1 (9.09)  | 31 (30.1)  | 0.18         |
| Epilepsy of unknown cause (n=30)                          | 7 (63.64)   | 23 (22.33)   | <b>0.007</b> |
| Epilepsy of unknown cause (focal)                         | 4 (36.36)   | 12 (11.65)   | <b>0.047</b> |
| Epilepsy of unknown cause (generalised)                   | 2 (18.18)   | 5 (4.85)   | 0.14         |
| Epilepsy of unknown cause (spasms)                        | 0 (0.0)   | 4 (3.88)   | 1.00         |
| Epilepsy of unknown cause (undetermined)                  | 1 (9.09)  | 2 (1.94)   | 0.26         |

### **3.4.2 Results of controls (n=65)**

Three out of 65 controls (4.61%) were positive for any of the tested antibodies including VGKC-complex Abs (n=2) and NMDAR Abs (n=1). None of the controls tested positive for CASPR2, LGI1 or GAD Abs.

#### ***3.4.2.1 Clinical features of the positive controls (n=3)***

The three positive controls were as follows:

1. Control 1 (VGKC 497pM): a 3 year old male with type 1 diabetes mellitus and no neurologic condition on four years follow up.
2. Control 2 (VGKC 200pM): a 2 year old male with vomiting illness and poor weight gain with no neurologic abnormality. He had no natural follow up recorded.
3. Control 3 (NMDAR score 2) : a 8 year old male with relapsed acute lymphocytic leukemia treated with bone marrow transplant which was complicated by chronic graft versus host disease. He had no neurological abnormality on four years follow up.

### **3.4.3 Comparison between positive seizure patients and positive controls**

11 out of 114 patients (9.65%) and 3 out of 65 controls (4.62%) were positive for any of the tested antibodies with no statistical significance ( $p=0.26$  Fisher's exact test). There was also no significant difference for the individual antibodies between patients and controls; see Table 3-8.

However when positive patients in the epilepsy of unknown cause category are compared to controls there is a significant difference (see section 3.4.1.5 above).

*Table 3-8 Comparison of the results of individual antibodies testing for patients and controls*

| <b>Antibody</b> | <b>Definition of positivity</b> | <b>Patients positive</b> | <b>Controls positive</b> | <b>P value</b> |
|-----------------|---------------------------------|--------------------------|--------------------------|----------------|
| <b>VGKC</b>     | >100 pM                         | 6/114                    | 2/62*                    | 0.71           |
| <b>LGI1</b>     | >1.0                            | 0/114                    | 0/65                     | 1.0            |
| <b>CASPR2</b>   | >1.0                            | 3/114                    | 0/65                     | 0.55           |
| <b>NMDAR</b>    | >1.0                            | 4/114                    | 1/65                     | 0.65           |
| <b>GAD</b>      | >100U/ml                        | 0/114                    | 0/65                     | 1.0            |
| <b>Gly R</b>    | >2.0                            | 0/114                    | 0/65                     | 1.0            |

\*62 tested for VGKC Abs due to samples exhaustion

### **3.5 Discussion**

#### **3.5.1 Methods and characteristics of cohort**

This study represents a cohort of children presenting with new onset seizures at a tertiary children hospital in Sydney, Australia. The cohort consists of a heterogeneous group of patients with epilepsy and epileptic seizures with a wide spectrum of severity and aetiology, including febrile seizures, acute symptomatic seizures due to various infectious and metabolic causes, epilepsies within the spectrum of defined electroclinical syndromes, epilepsies due to structural and metabolic causes, epilepsies caused by genetic defects and epilepsies of unknown cause. The patients were not selected according to the possibility of an autoimmune cause for their seizures (see inclusion criteria). Many patients in the cohort were young and complex, required intensive acute treatment or had ongoing refractory epilepsy as they represent an inpatient sample rather than a community outpatient sample.

The cohort description and classification represented a challenging exercise particularly at a time where the ILAE classification and terminology is evolving. The investigator(s) found the revised ILAE 2010 classification easy to use however in a few patients I needed to revisit the

previous classifications, such as for patients with "idiopathic generalised epilepsy". The investigator(s) performed the classification blinded to the results of antibody testing.

### **3.5.2 Results**

#### **3.5.2.1 Patients results**

The positive patients among the whole cohort were 11 out of 114 (9.65%), which is not different to adult studies examining epilepsy cohorts (McKnight et al., 2005, Brenner et al., 2013). However when the patients in our study were separated according to their epilepsy classification seven out of the 30 patients with epilepsy of unknown cause (23.3%) and four out of 16 patients with *focal* epilepsy of unknown cause (25%) were positive for neuronal antibodies.

The four patients with positive VGKC-complex Abs were negative for LGI and CASPR2, a finding that is similar to children previously reported with positive antibodies against VGKC-complex (Dhamija et al., 2011, Illingworth et al., 2011, Suleiman et al., 2011a) and different to adults where the target antigens in most patients with VGKC-complex antibodies were found to be LGI1, CASPR2 or contactin 2 (Irani et al., 2010a, Lai et al., 2010). It is likely that the VGKC-complex antibodies in children bind to other antigenic targets that are yet to be found or to the potassium channel itself. The four patients with positive VGKC-complex antibodies had epilepsy of unknown cause, and three had focal seizures at onset. The seizures were of temporal lobe onset in at least one patient (case 1).

In this cohort three patients tested positive for CASPR2 antibodies, which have not been identified in children before, however these patients tested negative for the VGKC complex antibodies in the radioimmunoassay and would have been missed with standard VGKC complex-Ab screening. The three patients with positive CASPR2 antibodies had focal seizures at presentation, with secondary generalisation in one.



None of the VGKC-complex or CASPR2 antibodies positive patients had a phenotype typical of limbic encephalitis, suggesting that neuronal antibodies can be present beyond the spectrum of these recognised syndromes, especially in children.

The two patients with positive NMDAR Abs had epilepsy in the absence of classic autoimmune NMDAR encephalitis. One patient with NMDAR Abs presented with focal dyscognitive seizures likely of occipital lobe origin, and one patient had a mixed seizure disorder and structural brain malformation. This is similar to adult cases described with new onset epilepsy associated with NMDAR Abs who also predominantly had extratemporal seizures (Niehusmann et al., 2009). Additionally, two patients, both presenting with generalised seizures, were positive for both NMDAR and VGKC-complex Abs; the finding of two or more neuronal Abs is well recognised (Pellkofer et al., 2010, Haberlandt et al., 2011, Irani et al., 2011b) and may reflect a wider activation of the immune system, likely by infections or possibly as a secondary response to neuronal damage.

The significance of the finding of two positive patients with known structural or metabolic cause for their epilepsy (case 7: MELAS and case 9: holoprosencephaly) is unclear and the antibody findings in these patients are likely to be either clinically irrelevant, or represent dual pathology. An association of neuronal surface (NMDAR) Abs has previously been reported in MELAS (Finke et al., 2012).

The investigations performed for the positive patients including CSF studies and brain imaging were unremarkable, a finding that is not uncommon in children with neuronal antibodies (Suleiman et al., 2011a). Only one patient out of four who had CSF studies had evidence of CNS inflammation evident by raised CSF neopterin (case 1). It is also interesting to note that 22 out of 55 patients tested had elevated CSF neopterin, a marker of CNS inflammation (Dale et al., 2009a). While some of those patients with positive neopterin had an inflammatory cause for their seizures (encephalitis or systemic infection), the others had different aetiologies and syndromes such as Dravet and West Syndromes and myoclonic

astatic epilepsy. It is possible that inflammation may play a role in a number of epilepsy syndromes, either as a primary phenomenon (such as autoimmunity), or as a secondary phenomenon (such as in neurodegeneration).

Nine of the 11 positive patients with recorded follow up had ongoing epilepsy but unfortunately, none of the 11 positive patients received immunotherapy. These patients did not develop long term cognitive or behavioural impairment although the follow-up was short and detailed neuropsychology was not done. This is in contrast to our previously described patients with VGKC-complex Abs who had poor outcome (Suleiman et al., 2011a). This may indicate that a wider range of seizure phenotypes is associated with neuronal antibodies including milder forms of epilepsy. One could hypothesise that immunotherapy, if given, might have improved the epilepsy outcome in these patients.

### ***3.5.2.2 Control results***

The control group consisted of children with various immunological, oncological and medical conditions and three controls out of 69 were positive for one of the tested antibodies although they did not have apparent neurological symptoms. This level of positivity is higher than in adult control cohorts (McKnight et al., 2005, Brenner et al., 2013). The ability to develop antibodies in response to circulating pathogens or environmental factors may be higher in children, particularly in those who are already unwell. It is possible that these positive controls could be at risk of future neurological dysfunction if the antibodies remain sustained, for instance during disruption of the blood brain barrier secondary to infection for example.

Two of the positive controls had conditions that can potentially result in immune disturbances namely type 1 diabetes mellitus (control1) and bone marrow transplant and immunosuppressant drugs (control 3). It is also possible that the presence of neuronal antibodies in these controls is an epiphenomenon or secondary to structural damage or immune activation, therefore results should be interpreted in the clinical context of the individual patient.

### **3.5.3 Limitations of the study and future directions**

This cohort represents a hospitalised, severe, young and co-morbid sample rather than a community or outpatient sample. The cohort would probably have been more antibody-positive if patients with a known cause for their epilepsy (such as structural and genetic) had been excluded, and if patients with other autoimmune diseases or patients with evidence of CNS inflammation had been positively selected (Suleiman et al., 2013a).

The significance of positive serum antibodies in the controls, and in some patients with a known alternate aetiology for their epilepsy is uncertain and raises some questions about the specificity of these serum antibodies in patients with seizures. CSF testing could possibly improve the sensitivity and meaning of the positive results (particularly for NMDAR) (Lancaster and Dalmau, 2012), however CSF testing was not performed in this study due to limited sample availability.

Further studies targeting patients with focal seizures of unknown cause might shed more light into the importance of these antibodies in this subgroup of patients with epilepsy. CSF testing of selected patients might help in understanding the significance of positive results. Future studies should also examine the effects of immune therapies in these patients.

It is important to note that finding a cause for epilepsy depends on how much investigations are performed and classifying patients into "epilepsy of unknown cause" category can be inaccurate particularly if investigations are incomplete or inadequate. Moreover epilepsy can be multifactorial and genetic defects can be associated with structural abnormalities (Shorvon, 2011).

## **3.6 Conclusions**

This study is the first to examine a relatively large cohort of children with new onset seizures and apply the latest ILAE classification and test them for neuronal antibodies.

The findings in this study suggest that a proportion of children with epilepsy of unknown cause might have neuronal antibodies as a cause for their epilepsy, in particular those with focal epilepsies. We suggest that antibody testing be part of the extended work up of these patients, in addition to other tests that are usually performed in this setting including high resolution imaging, genetic and metabolic studies. The current ILAE classification does not incorporate 'autoimmunity' in the aetiology or epilepsy classification. We suggest that an "immune or autoimmune" category is added to the list of structural metabolic causes in the epilepsy classification. Alternatively an additional aetiological category of "immune" or "inflammatory" may be added to the current list of genetic, structural metabolic and unknown causes. The fact that the role of these antibodies in the associated seizure and epilepsy syndromes is not fully understood and whether the immune system plays a primary or secondary role here might make the addition of this category a bit complicated.

# **Chapter 4 Case Series and Proposed Guidelines for the Identification of Autoimmune Seizures in Children**

## **Acknowledgment**

**This chapter is a slightly modified version of the published work in paper 3 in Appendix 6 (Suleiman et al., 2013a).**

## **4.1 Introduction**

The association between autoantibodies and central nervous system (CNS) disease is increasingly recognised. Serum and CSF antibodies that bind to neuronal cell surface proteins including channels and receptors have the potential to be pathogenic and cause CNS disease. By contrast onconeuronal antibodies are typically targeted against intracellular antigens and not thought to be directly pathogenic (Vincent et al., 2011a, Bien et al., 2012). Recently antibodies that bind extracellularly and are associated with CNS disorders have been called “neuronal surface antibodies” (NSAbs) and the disorders associated with these NSAbs are called “neuronal surface antibody syndromes” (NSAS) (Zuliani et al., 2012). There are well defined CNS syndromes associated with NSAbs where seizures are an important feature. Examples include NMDAR encephalitis in which 76 to 83 % of patients will have focal, focal dyscognitive or generalised seizures (Dalmau et al., 2007, Dalmau et al., 2008, Irani et al., 2010b, Dalmau et al., 2011, Irani and Vincent, 2011), and VGKC-complex antibody associated autoimmune limbic encephalitis (including LGI1 and CASPR2 antibodies) in which patients often have temporal lobe seizures (Irani et al., 2010a, Lai et al., 2010). In addition, faciobrachial dystonic seizures are seen in adults in association with LGI1 antibodies and often precede the onset of the limbic encephalitis (Irani et al., 2011b). Other NSAbs less frequently found in adults with limbic encephalitis are AMPA and GABA<sub>B</sub> receptor antibodies (Lai et al., 2009, Lancaster et al., 2010, Boronat et al., 2011). Antibodies to GAD have been associated with limbic encephalitis (Malter et al., 2010). Although GAD

is an intracellular antigen and therefore GAD Abs themselves may not be pathogenic, it is possible that unrecognised NSAbs may coexist with GAD Abs (Zuliani et al., 2012).

Zuliani et al proposed guidelines for the recognition, testing and treatment of suspected autoimmune CNS disorders. They used clinical criteria, supportive features, neuronal antibody testing and the response to immune therapy to classify patients into categories of definite, probable and possible NSAS, see Tables 4-1 and 4-2 (Zuliani et al., 2012).

Lancaster and Dalmau have proposed an alternative laboratory based algorithm for identification and assessment of antibodies to neuronal cell-surface antigens using cell based assays as well as rat brain immunohistochemistry and cultures of neurons for serum and CSF antibody binding (Lancaster and Dalmau, 2012).

*Table 4-1 Criteria and supportive features used in the work up for suspected NSAS (Zuliani et al., 2012).*

The following three clinical criteria are used to suggest a possible immune mediated cause associated with NSAbs

1. Acute or subacute (<12 weeks) onset of symptoms.
2. CNS inflammation manifested by at least one of:
  - a. CSF pleocytosis, oligoclonal bands or elevated IgG index;
  - b. MRI abnormality including increased signal in the mesiotemporal lobe (LE- like syndrome) and cerebellar enhancement (cerebellitis) or functional imaging abnormalities including hypermetabolism on positron emission tomography or hyperperfusion on single proton computed tomography;
  - c. inflammatory neuropathology on biopsy.
3. Exclusion of other causes (infection, trauma, toxic, metabolic, tumour, previous CNS disease).

The following supportive features would strengthen the suspicion for a suspected NSAS:

1. History of other antibody mediated condition (e.g myasthenia gravis) or organ specific autoimmunity.
2. Preceding infection, febrile illness or viral disease-like prodromes.

NSAbs: neuronal surface antibodies.

*Table 4-2 Classification diagnosis of suspected NSAS*

(Zuliani et al., 2012)

A diagnosis of definite, probable or possible NSAS is based on clinical presentation, NSABs testing and the response to immunotherapy, as follows:

A diagnosis of *definite NSAS* can be made if:

- known NSABs are present in serum or CSF
- AND there is response to immunotherapy.

A diagnosis of *probable NSAS* can be made if:

- known NSABs are present,
- OR there are other neuronal Ab markers (GAD, unknown neuronal surface or neuropil antibodies), or at least one of the supportive features above (history of other Ab mediated disorder or preceding fever/infection) are present AND there is a response to immunotherapy.

A diagnosis of *possible NSAS* can be made if clinical criteria are suggestive and no known NSABs are found, if:

- other neuronal Ab markers are present (GAD, unknown neuronal surface or neuropil),
- OR at least one of the above supportive features are present,
- OR there is a response to immunotherapy.

In children, NMDAR encephalitis is well described (Florance et al., 2009), whilst limbic encephalitis has been described in association with a number of different autoantibodies including VGKC-complex Abs (Haberlandt et al., 2011, Suleiman et al., 2011a). In children there are other epileptic conditions where immune-mediated mechanisms are suspected such as Febrile-Infection Related Epilepsy Syndrome (van Baalen et al., 2010) or Fever-Induced Refractory Epileptic Encephalopathy in School-aged children (Nabbout et al., 2010, Nabbout et al., 2011), both called FIRES. Previous terms used to describe similar syndromes include Devastating Epileptic Encephalopathy in School-aged Children (DESC) (Mikaeloff et al., 2006) and Acute Encephalitis with Refractory Repetitive Partial Seizures (AERRPS) (Sakuma, 2009). These conditions are characterised by new onset refractory focal status

epilepticus, preceded by fever or infection in previously normal children, followed by a chronic phase of refractory focal epilepsy and severe neurologic impairment (Sakuma et al., 2010). The cause of these conditions is unknown and underlying immune mechanisms have been proposed (Sakuma et al., 2010, Specchio et al., 2010, Nabbout et al., 2011).

Here we present a representative case series of 13 children suspected to have an autoimmune basis for their epilepsies. We propose modified guidelines for the recognition of autoimmune epilepsy and apply these guidelines to the 13 children with suspected autoimmune epilepsy to test their utility.

## **4.2 Methods**

### **4.2.1 Cases identification**

Through our clinical practice at The Children's Hospital at Westmead (CHW) we identified cases with seizures that may have an autoimmune aetiology. The neurology department at CHW is a busy tertiary children's hospital, which sees 300-400 children with new onset seizures per year, as well as other acute and chronic neurological diseases in children. The patients presented in this cohort were discussed in detail by JS and RCD as they were suspected to have an autoimmune cause of their epilepsy (as defined below), and were investigated for neuronal surface antibodies. This cohort does not represent all children with encephalitis (of all aetiologies) seen during this time (n=33) which are currently being studied in a separate research study.

It is also likely that other autoimmune epilepsies were missed by the investigating team and were not tested for antibodies. Instead this cohort should be considered a representative sample of children with suspected autoimmune epilepsy, which were used to test the utility of the modified guidelines.



We suspected autoimmune epilepsy in children with acute or subacute onset of seizures once other causes (infection, structural, metabolic or genetic) were excluded, and when any of the features described in Table 4-3 were present.

Here we describe 13 representative patients seen over a three and a half year period (late 2008 to mid 2012), who we suspected may have an autoimmune cause for their epilepsy, and who had serum available for testing for neuronal antibodies. No patients have been previously reported except case 5 (Suleiman et al., 2011a) and this case was included to test the utility of the guidelines. This study was approved by the hospital ethics committee.

10 patients had serum testing in the acute phase of their illness while in three the serum was from the chronic symptomatic phase. All samples were taken before immune therapy, if given. Antibody assays were all performed in Oxford UK using previously published methods (Irani et al., 2010a) apart from case 3, which was performed in National NMDAR antibody referral laboratory (Brisbane, Australia).

#### **4.2.2 Proposed modified guidelines**

In order to improve recognition and diagnosis of children with suspected autoimmune epilepsy, we modified the guidelines proposed by Zuliani et al for identification of children with neuronal surface antibodies syndromes (Table 4-3). Then, based on antibody testing and the response to immunotherapy (when given), we proposed five categories for classification (in descending order of likelihood of autoimmune epilepsy) including definite, probable, possible, unlikely or unknown autoimmune epilepsy (Table 4-4, Figure 4.1). We applied the modified guidelines to our 13 cases to test their usefulness (Table 4-5).

The main differences to the Zuliani et al guidelines for adults include the following:

- In children a paraneoplastic cause of epilepsy is very rare and testing for onconeural antibodies is rarely necessary. However; girls with positive NMDAR Abs should be screened for ovarian teratomas.

- In children fever and intercurrent infections are common and less likely to be a supportive feature for an autoimmune process as has been proposed in adults, so this was not used as one of the supportive features (Zuliani et al., 2012).
- We used elevated CSF neopterin as an additional marker of CNS inflammation (Dale et al., 2009a).
- In Zuliani et al., abnormalities on functional imaging including hypermetabolism on positron emission tomography or hyperperfusion on single photon computed tomography were used as features to suggest CNS inflammation. However there is inadequate research to demonstrate their ability to discriminate epilepsy aetiologies in children and therefore we did not include these features.
- Antibodies included in the guidelines are those available at international laboratories including antibodies against VGKC-complex proteins, LGI1 and CASPR2, NMDAR and GAD. Positive GAD antibodies in neurological disease is defined as >1000IU/ml. We did not include neuronal binding or neuropil antibody testing (using immunohistochemistry or immunofluorescence on rat brain tissue) for recognisable staining patterns (Lancaster and Dalmau, 2012) as these are done in research settings and are less available to clinicians routinely.
- Response to immunotherapy was defined as significant clinical improvement of encephalopathy or reduction of seizures as judged by the treating clinicians. We accept the subjective nature of this, and the fact that there are a number of confounders that could be responsible for improvements such as the concomitant change in anti-epileptic drugs.
- We incorporated patients who did not receive immunotherapy into the classification either because an immune mediated mechanism was not initially suspected or because of spontaneous improvement.

*Table 4-3 Criteria and supportive features to suspect autoimmune epilepsy*

*(modified from Zuliani et al.2012)*

The following two clinical criteria are used to suspect autoimmune epilepsy associated with NSAbs (both are needed)

4. Acute or subacute (<12 weeks) onset of symptoms.
5. Exclusion of other causes (CNS infection, trauma, toxic, tumour, metabolic, previous CNS disease).

The following supportive features would strengthen the suspicion of autoimmune epilepsy (patients should have at least 1 of the following):

- a. The presence of a well defined clinical syndrome such as NMDAR or limbic encephalitis
- b. CNS inflammation manifested by at least one of:
  - i. CSF pleocytosis (defined as >5 white cells/mm<sup>3</sup>) or presence of oligoclonal bands, elevated IgG index or elevated neopterin (defined as >30 nmol/L)
  - ii. MRI abnormality compatible with an inflammatory or autoimmune encephalitis including increased signal in the mesiotemporal lobe (LE-like syndrome)
  - iii. Inflammatory neuropathology on biopsy
- c. History of other antibody mediated condition (e.g. myasthenia gravis), organ specific autoimmunity or other autoimmune disorders.\*
- d. Improvement in clinical state after immunotherapy

\* It is recognised that epilepsy is more common in many autoimmune disorders including multiple sclerosis, systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), coeliac disease and autoimmune thyroid disease (Vincent and Crino, 2011).

NSAbs: neuronal surface antibodies.

*Table 4-4 Classification categories of suspected autoimmune epilepsy in children*

(identified using the criteria and supportive features in Table 4-3, modified from Zuliani et al).

Classification categories expressing the likelihood of autoimmune epilepsy based on the presence of NSAbs and GAD Abs and the response to immunotherapy (see also Figure 4.1):

*Definite autoimmune epilepsy* is present if:

- known NSAbs are present in serum or CSF
- AND there is response to immunotherapy

*Probable autoimmune epilepsy* is present if:

- known NSAbs are present and no immunotherapy responsiveness demonstrated (immunotherapy unsuccessful or not given)
- OR GAD antibodies are present AND there is response to immunotherapy

*Possible autoimmune epilepsy* is present if known NSAbs are negative and:

- GAD antibodies are present and no immunotherapy responsiveness demonstrated (unsuccessful or not given)
- OR GAD antibodies are negative and there is a response to immunotherapy

*Unlikely autoimmune epilepsy* is present if:

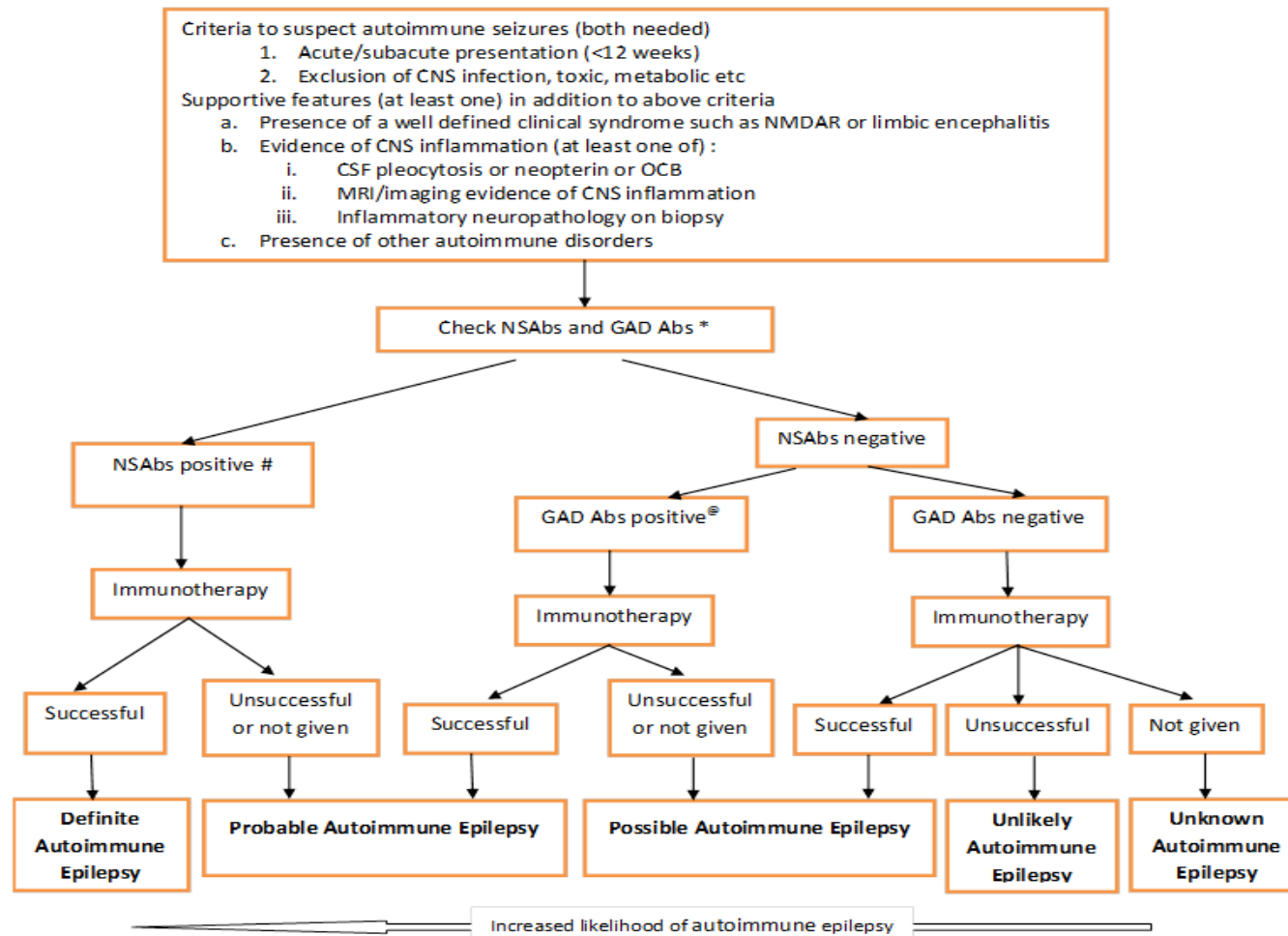
- known NSAbs and GAD are negative and there is no response to immunotherapy

*Unknown autoimmune epilepsy\** is present if:

- known NSAbs and GAD are negative and immunotherapy is not given

\* Patients in this category may move to a different category if they receive immunotherapy, such as “possible” if they respond or “unlikely” if they did not respond to immunotherapy.

Figure 4.1 Flow chart for approach to children with seizures of suspected autoimmune aetiology



OCB: oligoclonal bands, \*: onconeural Abs testing is rarely necessary in children, #: if NMDAR look for ovarian teratoma in females, @: GAD positivity is defined as >1000u/ml. Immunotherapy refers to high dose steroids and/or intravenous immunoglobulins. (Flow chart modified from Zuliani et al., 2012).

*Table 4-5 Patients with suspected autoimmune epilepsy: clinical criteria, supportive features and classification*

*(as per guidelines, see Figure 4.1)*

| Case | Age (years)/sex | Epilepsy diagnosis  | Acute or sub acute onset | Seizure type                                     | Associated features  | CSF inflammation (pleocytosis/ OCB/ neopterin) | MRI inflammatory changes | Presence of autoimmune / Ab mediated disease | NSAbs/ GAD Abs      | Response to immune therapy        | Outcome                           | Guidelines classification |
|------|-----------------|---------------------|--------------------------|--|--|--|--------------------------|--|---------------------|-----------------------------------|-----------------------------------|---------------------------|
| 1    | 3/F             | NMDAR encephalitis  | +                        | Focal dyscognitive                               | Encephalopathy, aphasia, dystonia, emotional lability, relapse | -/+ / +  | -                        | -  | NMDAR CSF and serum | + (steroid, IVIG, mycophenolate ) | Relapse, normal in between        | Definite                  |
| 2    | 6/M             | NMDAR encephalitis  | +                        | Focal dyscognitive                               | Encephalopathy, agitation, chorea, dystonia                    | +/-/+  | -                        | -  | NMDAR CSF and serum | + (steroid and IVIG)              | Recovery                          | Definite                  |
| 3    | 7/F             | NMDAR encephalitis  | +                        | Focal dyscognitive                               | Encephalopathy, agitation, irritability, dyskinesia, fever     | +/- /ND  | +                        | -  | NMDAR CSF and serum | + (steroids, IVIG)                | Recovery                          | Definite                  |
| 4    | 1/F             | VGKC encephalitis   | +                        | Focal dyscognitive , focal motor with automatism | Encephalopathy, fever, respiratory infection                   | + /ND/+  | +                        | -  | VGKC serum (421 pM) | Not given                         | Recovery                          | Probable                  |
| 5    | 15/F            | VGKC encephalitis   | +                        | Focal, secondary generalised tonic clonic, SE    | Encephalopathy, memory deficit, fever                          | +/-/+  | -                        | -  | VGKC serum (640 pM) | + (steroids, IVIG)                | Relapse, normal in between        | Definite                  |
| 6    | 12/F            | Limbic encephalitis | +                        | Focal dyscognitive                               | Encephalopathy, lethargy, behavioural alteration               | ND   | +                        | +/- (ANA)                                    | negative            | Not given                         | Cognitive, psychiatric impairment | Unknown                   |

|    |      |                                     |   |  |  |         |     |                              |                     |                               |   |          |
|----|------|-------------------------------------|---|--|--|---------|-----|------------------------------|---------------------|-------------------------------|---|----------|
| 7  | 15/F | Limbic encephalitis                 | + | Focal dyscognitive, secondary generalised tonic clonic | Encephalopathy, cognitive deficits, fever  | +/-/+   | -   | -                            | negative            | + (steroid)                   | Recovery  | Possible |
| 8  | 3/M  | FIRES                               | + | Focal, status epilepticus                              | Encephalopathy, irritability, fever, rash, | -/-/+   | -   | -                            | negative            | - (steroids, IVIG, rituximab) | Severe neurologic disability, refractory epilepsy | Unlikely |
| 9  | 8/F  | FIRES                               | + | Focal, secondary generalised                           | Encephalopathy, headache, confusion, fever | +/-/+   | +/- | -                            | negative            | - (steroids)                  | Severe neurologic disability, refractory epilepsy | Unlikely |
| 10 | 1/F  | Epileptic encephalopathy            | + | Epileptic spasms                                       | Encephalopathy, developmental delay        | -/+/+   | -   | -                            | VGKC serum (201 pM) | + (steroids)                  | Developmental delay                               | Definite |
| 11 | 13/F | Suspected autoimmune epilepsy (JME) | + | Myoclonic, generalised tonic clonic                    | Hyperthyroidism                            | ND      | -   | + (Grave's disease and T1DM) | negative            | Not given                     | Ongoing epilepsy                                  | Unknown  |
| 12 | 3/F  | Suspected autoimmune epilepsy       | + | Focal dyscognitive                                     | Myasthenia                                 | ND      | -   | + (MG)                       | negative            | + (steroids)                  | Steroid dependent myasthenia                      | Possible |
| 13 | 4/F  | Suspected autoimmune epilepsy       | + | Absence  | Ataxia                                     | -/ND/ND | No  | + (T1DM)                     | GAD (3000U/ml)      | Not given                     | Cognitive impairment, ongoing epilepsy            | Possible |

Definitions: FIRES: Fever-Induced Refractory Epileptic Encephalopathy in School-aged children, SE: status epilepticus, JME: Juvenile Myoclonic Epilepsy, OCB: oligoclonal bands, ND: not done, T1DM: type 1 diabetes mellitus, MG: myasthenia gravis, NSAb: neuronal surface antibody, Encephalopathy is defined by the presence of acquired reduction in consciousness, cognitive dysfunction or behavioural change lasting more than 24 hours, and not related to the post-ictal state

+: present or positive, -: absent or negative

Abnormal ranges: Pleocytosis: CSF white blood cells > 5cells/mm<sup>3</sup>, Neopterin elevated >30 nmol/L, VGKC serum >100pM, High titre cut-off for GAD antibodies in neurological disease is 1000U/ml.

### 4.3 Results

The 13 patients with seizures of suspected autoimmune origin (11 females, age range 1-13 years, mean 6 years) are presented in Table 4-5. All patients had other potential causes for their seizures excluded. All 13 patients had new onset seizures and at least one supportive feature of CNS inflammation or the presence of other autoimmune diseases (Table 4-3 and 4-4). Three patients had the clinical characteristics of NMDAR encephalitis (all with CSF and serum NMDAR antibodies), two had encephalitis associated with VGKC-complex antibodies, two had features suggestive of limbic encephalitis (with negative antibodies), three had epilepsy in association with other autoimmune diseases (one with GAD antibodies), two had FIRES, and one had epileptic encephalopathy with CNS inflammation (VGKC antibody positive).

Seven patients out of the 13 (53.9%) were positive for one of the tested antibodies including NMDAR (n=3), VGKC-complex (n=3) and GAD (n=1). Immunotherapy was given in nine patients, five with positive neuronal antibodies, and four negative. The immune therapy was steroids alone (n=4), steroids and IVIG (n=3), steroids, IVIG and mycophenolate (n=1) and steroids, IVIG and rituximab (n=1). All five patients with positive neuronal antibodies who received any immune therapy improved after receiving therapy, whereas only 2 of 4 with negative neuronal antibodies improved after receiving therapy. Three out of the four patients who did not receive immunotherapy had poor outcome including ongoing epilepsy, cognitive and psychiatric impairment (Table 4-5).

Patients were classified according to the proposed modified guidelines (Table 4-4, figure 4.1) and their classification is presented in Table 4-5. Five patients had definite, one had probable, three had possible, two had unlikely and two had unknown autoimmune epilepsy.

We present the case histories for nine of the 13 patients in detail below as representative examples.



## 4.5 Case histories

### 1) Case 3: NMDAR encephalitis

A previously well seven year old girl had a prodromal illness of headache, fevers, vomiting and abdominal pain followed by right leg pain and difficulties with fine motor skills. Memory impairment and cognitive decline followed. Three weeks into her illness she developed an absence-like seizure with unresponsiveness and salivary drooling that lasted for 10 minutes. She had more episodes suggestive of focal dyscognitive seizures and was admitted to hospital, and treated with phenytoin and levetiracetam. She developed abnormal movements in the form of orobuccal dyskinesia, excessive blinking, non purposeful limb movements and excessive thrashing movements in her bed. Her behaviour was altered with agitation, irritability, inappropriate affect and mood swings. She had slurred and reduced speech and became confused and disoriented.

Neuropsychological assessment showed significant impairment in language and verbal intellect as well as mild impairment in processing speed and fluctuation in attention. EEG showed left temporal slowing and no epileptiform activity. CSF analysis revealed 15 white cells/mm<sup>3</sup> and MRI revealed hyperintensities in the right frontal, left temporal and insular cortex. Ovarian ultrasound was normal. NMDAR Abs were positive in serum and CSF and VGKC-complex Abs were negative.

She was treated with intravenous pulse 30 mg/kg/day methylprednisolone for five days followed by oral prednisolone weaning regimen over four weeks in addition to intravenous immunoglobulin at a dose of 2g/kg. Three weeks later she was reported by her family to be 90% back to normal and had no ongoing seizures or abnormal movements. Further doses of monthly IVIG over 3 months were given and associated with a full recovery.

This patient had positive NMDAR antibodies and positive response to immunotherapy and applying the guidelines' classification she has "definite" autoimmune epilepsy.

### 2) Case 4: VGKC-complex Abs associated encephalitis

A previously well 15 month old girl presented after an upper respiratory tract infection with a 30 minute new onset focal seizure involving the left side of the face and the left arm. At the hospital she had a low grade fever and was floppy, unresponsive and staring into space. She had more episodes of focal seizures consisting of facial twitching, staring and lip smacking. Seizures were treated with phenytoin, phenobarbitone and midazolam and stopped within five days of onset. Encephalopathy, cognitive or behavioural alterations were not prominent features of her illness once the seizures were controlled.

EEG showed diffuse slowing of the background with superimposed fast activity and no epileptiform activity. CSF analysis revealed 6 white cells/mm<sup>3</sup> and raised neopterin of 205 nmol/L (normal <30). CSF culture and viral PCR were negative. Mycoplasma IgM was positive using complement fixation test and there was no hyponatremia. MRI of the brain revealed T2 high signal in right basal ganglia, both temporal lobes and in the right parietal lobe. Testing of her serum from the acute illness revealed positive VGKC-complex Abs at 421 pM (normal <100pM) but negative antibodies against CASPR2, LGII, NMDAR and GAD.

Four months following her illness she was back to normal and continued to achieve developmental milestones appropriate to her age. She had no further seizures and her anti-epileptic drugs (AEDs) have been withdrawn.

This patient was positive for a known NSAb (VGKC-complex Ab) but did not receive immunotherapy therefore she has “probable” autoimmune epilepsy.

### **3) Case 6: Limbic encephalitis with negative NSAbs**

A previously well 12 year old girl presented to the local hospital with frequent “funny episodes” over six week period. The episodes started with nausea followed by confusion and disorientation, and lasted 60-90 seconds. She had lethargy, intermittent headache and behaviour alteration with episodes of agitation, screaming and confusion. The episodes were initially thought to be psychological until an EEG performed four weeks after discharge showed epileptiform activity in the right temporal region and MRI brain then showed swelling in the right hippocampus . She was commenced on

carbamazepine and referred to our hospital. She had ongoing temporal lobe seizures when she was reviewed at our hospital a few months after her acute illness. Repeated MRI scan at this time showed resolution of the right temporal lobe swelling and it was concluded that she had limbic encephalitis. Academic decline and emotional and behavioural abnormalities were reported, and neuropsychological testing showed difficulties in higher level thinking skills, attention, behaviour and emotional function.

Three years following her acute illness the seizures have settled and carbamazepine was stopped. Repeat neuropsychological assessment showed persistent cognitive impairment. Autoimmune screen showed very high ANA titres at 1: 2560 (normal <1:40) with a strong centromere staining pattern, although further immunological investigations and clinical assessments failed to confirm a diagnosis of systemic autoimmune disease. Antibodies against VGKC-complex, NMDAR and GAD tested on serum collected three years after onset of her seizures were negative.

This patient illness is consistent with autoimmune limbic encephalitis based on clinical presentation, MRI abnormality, and the associated high titre of ANA which supported an autoimmune tendency.

Despite the clinical suspicion of limbic encephalitis, using the classification she has “unknown” autoimmune epilepsy as she was NSAbs and GAD Abs negative and received no immunotherapy.

#### **4) Case 8: Fever-Infection Related Epilepsy Syndrome- FIRES**

A previously well three year old boy presented with fever and blanching rash for a few days followed by irritability and reduced level of consciousness. He was semi-comatose on presentation to hospital and required intubation and ventilation. He had status epilepticus with frequent and prolonged focal seizures which were refractory to AEDs.

CSF analysis revealed 4 white cells/mm<sup>3</sup> and elevated neopterin at 264 nmol/L (normal <30).

Extensive serological, microbial, metabolic and genetic testing did not reveal aetiology. EEG showed diffusely slow background with quasiperiodic multifocal high voltage epileptiform activity followed by periods of electrical attenuation and frequent multi-focal electrical and clinical seizures. MRI was

normal apart from nonspecific signal abnormality in the white matter of the frontal and occipital lobes. Subsequent MRI showed loss of white matter volume and increased signal in the periventricular areas. Antibodies against VGKC-complex, LGI1, CASPR2, NMDAR and GAD were negative.

Intensive care treatment with multiple antiepileptic drugs and barbiturate induced coma as well as ketogenic diet did not help control his seizures. Early immunotherapy in the form of high dose intravenous pulse methylprednisolone at 30 mg/kg/day for three days followed by oral prednisolone for eight weeks and adjunctive intravenous immunoglobulin 2 g/kg followed by anti-CD20 antibody rituximab had no effect.

After 64 days in intensive care he was discharged to the ward, and continued to have refractory focal seizures and significant neurological impairment. He was discharged home after eight months.

Sixteen months later he had severe cognitive and developmental impairment, refractory focal epilepsy on six AEDs and prolonged hypogammaglobulinemia presumed secondary to rituximab treatment and requiring IVIG replacement.

#### **5) Case 9: FIRES**

A previously well eight year old girl presented with high fever, headache and lethargy, confusion with altered level of consciousness and proceeded to have frequent focal seizures which evolved into generalised seizures requiring intubation and ventilation.

CSF analysis revealed 5 white cells/mm<sup>3</sup> and elevated neopterin at 296 nmol/l (normal <30) but negative oligoclonal bands, viral PCRs and bacterial cultures. Extensive work up for infectious agents, immune screen and metabolic investigations were negative. Initial EEG showed diffuse high-voltage slowing consistent with encephalopathic process. Subsequent EEGs showed burst suppression, multifocal epileptiform activity and focal electrical seizures alternating from both hemispheres. Initial MRI was normal and subsequent acute MRIs revealed meningeal enhancement, patchy areas of cerebral oedema and high signal in bilateral thalami and hippocampi. Subsequent MRI showed global cerebral and cerebellar atrophy and periventricular white matter high signal.

Intensive care treatment with mechanical ventilation, thiopentone and multiple antiepileptic drugs did not control her seizures. She received ketogenic diet and electroconvulsive treatment (ECT) without effect. She was given high dose intravenous methylprednisolone 30 mg/kg/day over 3 days with negative response. No other forms of immunotherapy were given.

After eight months in intensive care she was discharged to the ward where she continued to have multiple daily focal seizures. She was discharged home after 20 months in hospital in vegetative state. Four years after her illness she remained in “minimally conscious state” with refractory focal seizures on multiple AEDs.

Retrospective testing of serum stored from the acute illness for antibodies against VGKC-complex, LGI1, CASPR2, NMDAR and GAD were negative.

Using the classification these two cases with FIRES have “unlikely” autoimmune epilepsy as they are negative for known NSAbs and GAD Abs and were unresponsive to immune therapy.

#### **6) Case 10: Epileptic encephalopathy with evidence of CNS inflammation**

This case was reported and published (Suleiman et al., 2011b), see Appendix 6, here we present a summary of her case history to apply the guidelines.

A 13 month old girl was referred to our hospital with epileptic spasms with onset at four months of age and significant developmental delay. EEG showed slow background with high amplitude multifocal spike and slow-wave complexes compatible with “modified hypsarrhythmia” and consistent with epileptic encephalopathy. Brain MRI showed mild diffuse cerebral atrophy and mild delay in white matter myelination. Extensive metabolic testing was negative. CSF had no cells, but CSF neopterin was mildly elevated at 33.3nmol/L (normal<28) and mirrored oligoclonal bands (OCBs) were detected in CSF and serum.

Treatment with vigabatrin, pyridoxine, biotin and levetiracetam had minimal or no benefit. Over the next few months, the patient had no obvious clinical seizures; however, she made no progress in her development. She lived internationally and was two years old at the time of reassessment. Her EEG

remained encephalopathic with a modified hypsarrhythmic pattern; therefore oral prednisolone at 40mg daily was started and was followed by improvement in the encephalopathic state with improved awareness and interactions noted by the family. Repeat EEG eight days after starting steroids showed improvement in hypsarrhythmia.

Retrospective testing of serum taken at 13 months of age was positive for VGKC-complex (201pmol/L, normal < 100pmol/L), but negative for LGI1, and CASPR2, NMDAR and GAD antibodies.

This patient had positive VGKC-complex Abs and positive response to immunotherapy therefore she has “definite” autoimmune epilepsy.

#### **7) Case 11: Epilepsy, type 1 diabetes mellitus (T1DM) and autoimmune thyroid disease**

A 13 year old girl with well controlled T1DM presented with early morning episodes of shaking lasting a few minutes and often followed by confusion and fatigue. She developed early morning myoclonic jerks in the absence of hypoglycaemia and some of the episodes were associated with loss of consciousness. At this time she was noted to have recent loss of weight and had tachycardia, hypertension and goitre on examination.

Thyroid function tests were consistent with hyperthyroidism. Thyroid antibodies were elevated including anti thyroglobulin antibodies 1314 U/ml (<60), anti thyroid peroxidase antibodies 105 U/ml (<35) and anti thyrotropin receptor antibodies 35.7 U/L (0 – 2). EEG showed generalised spike and wave discharges suggestive of idiopathic generalised epilepsy but MRI brain was normal.

She was diagnosed with Graves’ disease and juvenile myoclonic epilepsy (JME), commenced on carbimazole for the treatment of thyrotoxicosis and her epilepsy was successfully treated with AEDs.

Retrospective testing of her serum collected at the time of seizure onset was negative for VGKC-complex, LGI1, CASPR2, NMDAR and GAD antibodies.

This child had negative NSAbs and GAD Abs and received no immunotherapy and applying the guidelines she has “unknown” autoimmune epilepsy.

### **8) Case 12: Anti MuSK myasthenia gravis and epilepsy**

A previously healthy three year old girl presented with a four month history of ptosis and non-conjugate eye movements with diurnal variation. Examination confirmed a fatigable ptosis and variable ophthalmoplegia, but no generalised muscle weakness. Mestinon test was associated with clinical improvement confirming the diagnosis of myasthenia. Serum AChR antibodies were negative however MuSK antibodies were positive with a titre of 0.87 nmol/L (normal <0.09). At the same time as the myasthenia she started to have staring episodes, occurring multiple times per day. Her EEG showed bilateral occipital epileptiform discharges (L>R) without photosensitivity consistent with a focal epilepsy. Her MRI brain was normal and autoimmune screen was negative. Antibodies against VGKC-complex, NMDAR and GAD were negative.

She was treated with carbamazepine and low dose oral prednisolone at 5 mg daily for four weeks with partial improvement then increased to 2 mg/kg/day with complete resolution of myasthenic and epileptic symptoms. Initial attempt to wean off steroid was associated with recurrence of ocular symptoms as well as seizures while she remained on AED thus steroids were maintained. After 17 months of disease, she is steroid dependent despite adding azathioprine, but her ocular myasthenia gravis and seizures are in remission. Long term steroid treatment was associated with behavioural adverse side effects.

Both the patient's epilepsy and myasthenia gravis responded to immunotherapy particularly steroids. She was negative for NSAbs and GAD Abs and applying the guidelines to this case she has "possible" autoimmune epilepsy.

### **9) Case 13 T1DM, Epilepsy, ataxia and high titre GAD antibodies**

A 4 year old girl with chronic ear infection, grommets, hearing impairment and speech delay presented with acute unsteadiness of gait, lethargy and irritability. She was febrile and had a rash thought to be consistent with a viral exanthem. She had difficulty obeying commands, was ataxic and had mild hand tremor; but no other focal neurologic findings.

MRI brain was normal and EEG showed paroxysmal epileptic discharges during sleep. CSF analysis showed no cells, normal glucose, protein and lactate and negative culture. Her symptoms recovered during her hospital stay over 5 days however she was noted to have infrequent staring episodes at the time of discharge. Her illness was thought to be consistent with an immune mediated ataxia with complete recovery.

Five years following her acute ataxia she presented with new onset T1DM. She was reassessed by her neurologist for concerns about ongoing absence episodes that were associated with eye deviation, and thought to be consistent with focal seizures. However, the events were infrequent and required no treatment. The child had cognitive impairment and was attending a support class at school. EEG was normal and Video EEG was not performed. Testing of serum collected at the time of onset of DM showed highly elevated GAD antibody titre at 3000 U/mL and negative antibodies against VGKC-complex, LGI1, CASPR2 and NMDAR.

Applying the guidelines this patient has “possible” autoimmune epilepsy as she was negative for NSAbs but positive for GAD Abs and received no immunotherapy.

## **4.6 Discussion**

The recognition of immune mechanisms in neurological disorders is important as this can prompt early treatment and may lead to better outcomes. The identification of specific and potentially pathogenic NSAbs is increasing and the spectrum of the clinical syndromes associated with NSAbs is widening (Zuliani et al., 2012). Recently guidelines have been developed to help in the diagnosis and management of adults with suspected NSAS (Zuliani et al., 2012). In children the lack of large studies regarding NSAbs and their related syndromes makes it harder to identify these cases, therefore guidelines may help in the identification of NSAS particularly when seizures are an important feature.

In this study, we describe 13 representative patients with seizures of suspected autoimmune aetiology and we propose features for identification of these paediatric patients, and a classification system



testing the strength of evidence of autoimmune epilepsy based on the presence of neuronal antibodies and response to immunotherapy.

There were some general features common to the cohort. Females were over-represented in this cohort, as is often described in autoimmune disorders in general. The seizures were often focal, and generally occurred in association with encephalopathy or other features of CNS dysfunction.

Three cases had typical features of NMDAR encephalitis in children, as represented by case 3 description. The NMDAR encephalitis patients generally had focal epilepsy, and the presence of psychiatric manifestations, behaviour alteration and movement disorder were strong indicators of NMDAR encephalitis. However it is possible that NMDAR Abs are present in children with epilepsy in the absence of the classic phenotype as has been described in adults (Niehusmann et al., 2009), and therefore testing for NMDAR Abs in children with suspected autoimmune seizures may provide further information about the spectrum of NMDAR antibody associated disease.

Two cases had VGKC-complex Ab associated encephalitis, characterised by fever associated focal seizures and status epilepticus, one was previously reported in chapter 2 (case 5) and the clinical phenotype of the second newly reported case (case 4) was similar to our previously reported paediatric patients with VGKC-complex Abs associated encephalitis (Suleiman et al., 2011a). The seizure semiology was suggestive of temporal lobe onset, a finding that is commonly seen in both adults and children with this syndrome (Vincent et al., 2004, Suleiman et al., 2011a). In Case 4, Mycoplasma IgM was positive and was consistent with acute infection. Mycoplasma infection has been described in association with NMDAR encephalitis in children and may be a trigger of autoimmune CNS disorders (Florance et al., 2009). However, mycoplasma pneumoniae is a common cause of respiratory infections in children and positive mycoplasma serology may therefore be incidental in some patients (Waites and Talkington, 2004). Antibodies against LGI1 or CASPR2 which have been identified as the target of VGKC-complex Abs in adults were negative in this case, a finding that is common in children with positive VGKC-complex Abs. It is possible that in children VGKC-complex Abs are targeted against other antigens in the VGKC-complex that are yet to be

identified. Patients with VGKC-complex Ab associated encephalitis often respond to immune therapy but spontaneous improvement can also occur (Irani et al., 2010a) as was the case in this patient.

Case 6 had a syndrome of limbic encephalitis however NSAbs and GAD Abs were negative possibly due to late testing and the patient received no immunotherapy, her classification is "unknown". Early recognition, testing and treatment might have improved her outcome. Case 7 had a limbic encephalitis syndrome and was negative for NSAbs but responded to immune therapy and her classification is "possible". Antibodies against AMPAR and GABA<sub>B</sub>R (not tested) or other unrecognised NSAbs could be the cause of limbic encephalitis in these patients. The diagnosis of limbic encephalitis can be challenging in children, where its existence is reported but probably under-recognised (Haberlandt et al., 2011). The diagnosis of limbic encephalitis is partly clinical with new onset temporal lobe seizures and cognitive disturbance, sometimes associated with radiological mesial temporal or hippocampal changes. As hippocampal signal change is described in a proportion of children with febrile status epilepticus (Shinnar et al., 2012), it is difficult to discriminate radiologically seizure induced hippocampal swelling from limbic encephalitis.

Cases 8 and 9 were typical of "FIRES" (van Baalen et al., 2010, Nabbout et al., 2011). Neuronal antibodies were negative and there was no response to immunotherapy in both patients. The absence of antibodies and the negative response to immune therapy make an autoimmune aetiology "unlikely". Negative response to immunotherapy has been reported in a series of seven cases of FIRES, and NSAbs were negative in the tested patients (three tested for VGKC-complex Abs and one for NMDAR Abs) (Howell et al., 2012). In addition a series of 12 children with FIRES were negative for neuronal surface antibodies and GAD (van Baalen et al., 2012). There is one report of a boy with positive VGKC antibodies associated with FIRES who benefited from immunotherapy (Illingworth et al., 2011), however this case did not have a typical course of FIRES and it is possible that the case had VGKC-complex antibody associated encephalitis instead. The markers of CNS inflammation seen in our two cases (8 and 9) have been reported in the acute phase of FIRES, and may be explained by the extremely high seizure load, seizure-related neuronal injury or cytokine release (Howell et al., 2012).

Rather than an autoimmune epilepsy syndrome, FIRES may be a genetic channelopathy or a chronic epilepsy syndrome with explosive onset (Ismail and Kossoff, 2011, Howell et al., 2012).

Case 10 had epileptic encephalopathy and epileptic spasms. This patient had positive VGKC-complex Abs and positive response to immunotherapy and “definite” autoimmune epilepsy when we applied the guidelines. However steroid responsiveness exists in patients with epileptic spasms even in the absence of antibodies or immune mediated aetiologies.

Three of our cases had epilepsy in association with other autoimmune diseases including Type 1 diabetes mellitus (T1DM) and autoimmune thyroid disease (case 11), anti MuSK myasthenia gravis (case 12), and T1DM and possible autoimmune ataxia (case 13). T1DM is a T cell mediated autoimmune disorder and there is an increased prevalence of epilepsy in children with this disease (Schober et al., 2012). Seizures can occur in Hashimoto’s encephalopathy, which is a rare association of autoimmune Hashimoto’s thyroiditis associated with Abs against thyroid peroxidase and thyroglobulin (Castillo et al., 2006). Patients described with Hashimoto encephalopathy present with broad clinical manifestations and are classically reported to be steroid responsive. The role of thyroid antibodies in Hashimoto encephalopathy is uncertain and the term “Steroid responsive encephalopathy associated with autoimmune thyroiditis” (SREAT) has been used to reflect the hypothesis that Hashimoto encephalopathy may be caused by unidentified neuronal autoantibodies (Castillo et al., 2002, Schauble et al., 2003).

Graves’ disease is an antibody mediated autoimmune disorder and JME has been previously associated with Grave’s disease, and may be due to thyroxine causing a lower seizure threshold (Su et al., 1993). Our case 11 was diagnosed to have juvenile myoclonic epilepsy (JME) based on her age, seizure phenotype and EEG abnormality. JME is considered to be a genetic epilepsy, and indeed in this case there was limited evidence that the epilepsy was autoimmune despite the presence of other autoimmune diseases, and her classification was “unknown” as she was negative for NSAbs and received no immunotherapy.

Seizures in association with anti MuSK Ab myasthenia gravis are rare but have been reported in an adult patient (Bhagavati et al., 2007) . Case 12 had anti-MuSK Ab associated myasthenia gravis and concurrent focal epilepsy. Her seizures did not respond to carbamazepine but improved when high dose steroids were used to treat her myasthenia gravis. It is possible that myasthenia gravis and epilepsy in our patient is a chance association, although both clinical entities presented, remitted and relapsed concurrently.

Case 13 had seizures in the context of T1DM. This patient had an acute transient ataxia followed by chronic epilepsy, with very high GAD antibodies. GAD antibodies are associated with a variety of CNS syndromes including stiff person syndrome, immune ataxia, epilepsy and limbic encephalitis (Honnorat et al., 2001, Saiz et al., 2008, Malter et al., 2010). In our patient the immune mediated ataxia, cognitive impairment, focal epilepsy and high GAD antibodies were supportive of the autoimmune epilepsy hypothesis.

Patients with epilepsy and other systemic autoimmune diseases may have other as-yet-unidentified NSAbs. However other explanations for increased epilepsy incidence in systemic autoimmune disorders include incidental coexistence, a common genetic predisposition, or secondary effects of the primary disease (Vincent and Crino, 2011).

One important feature of the adult guidelines is that response to immunotherapy is used as a retrospective feature to help with classification. In other words the “guideline classification” cannot be completed until immunotherapy is used. Our modified guidelines partly address this issue and incorporate patients who did not receive immunotherapy. In our case series some patients did not receive immunotherapy either because an autoimmune aetiology was not initially suspected at presentation or due to spontaneous improvement without the need for immunotherapy. A positive response to immunotherapy was more common in patients who had positive NSAb (five out of five given immunotherapy) compared to those who were NSAb negative (two out of four). However, in a recent study of 48 children with suspected autoimmune encephalitis, only 21 had specific antibodies

detected, and beneficial treatment responses were seen in both antibody positive and negative groups (Hacohen Y, 2013).

In our clinical practise over the last few years we have been increasingly using immunotherapy empirically once an underlying immune-mediated disorder is suspected whilst awaiting the specific investigations. Children suspected of potential autoimmune epilepsy undergo investigations to exclude infectious, toxic, metabolic or genetic causes, and neuronal surface and GAD antibodies are requested. Whilst awaiting the results of the neuronal antibodies, empiric immunotherapy may be commenced if the clinical syndrome is severe and impairing. We suggest that immunotherapy be used early in the disease course to optimise its potential effect. The regimen we have been using includes intravenous pulse methylprednisolone at 30 mg/kg/day for three days followed by a tapering course of oral prednisolone (variable duration of weeks to months according to the disorder), often in conjunction with intravenous immunoglobulins at 2 g/kg given over two days. Patients with partial response or no response after one to three weeks may receive further doses of intravenous immunoglobulins or plasma exchange if the condition is severe and concerning, and the autoimmune hypothesis remains possible. Patients who fail to respond or who have a partial response may be considered for second line therapy, such as rituximab or cyclophosphamide. However the side effect profile of these drugs is more concerning so a “risk versus benefit” assessment is necessary. In our case series immunotherapy was generally tolerated well particularly when given short term (such as the NMDAR encephalitis cases). Two patients developed significant side effects attributed to immunotherapy including behavioural alteration with prolonged steroid use (case 12) and prolonged hypogammaglobulinemia requiring IVIG replacement presumed to be secondary to rituximab (case 8), a finding that has been previously described (Makatsori et al., 2012). Some patients with seizures of autoimmune aetiology can have complete recovery without immunotherapy (similar to case 4); however it is hard to predict which cases will spontaneously recover and therefore early immunotherapy is suggested when the patient is severely impaired. Similar treatment regimens have been used in adults with VGKC Ab positive encephalitis with good effect (Reid et al., 2009, Wong et al., 2010). Although plasma exchange is used commonly in adults, the use of plasma exchange in

children as a modality of immune therapy is limited due to its invasiveness, the need for intensive care treatment and potential side effects.

Although a positive response to immunotherapy supports immune mediated mechanisms, steroids (and IVIG to a lesser extent) are used in the treatment of refractory and severe epilepsies that are not proven to be autoimmune.

In conclusion autoimmune mechanisms play an important role in a proportion of children presenting with seizures. We propose guidelines that may help clinicians in the approach to identify children with suspected autoimmune seizures. Although helpful, the guidelines are not perfect and only represent an attempt to identify and classify these patients. These guidelines do not predict treatment responsiveness or outcome. Future studies may improve the understanding of clinical phenotypes of autoimmune epilepsy in children and help further develop syndrome-specific and treatment oriented guidelines.

## **Chapter 5 Conclusions and Future Directions**

Here I summarise the findings in each of the studies and discuss the limitations of these studies as well as the potential directions for future research targeting autoimmune seizures in children.

### **5.1 Autoimmune VGKC encephalitis**

The first study (see Chapter 2) aimed to investigate neuronal antibodies in children presenting with status epilepticus in the context of unexplained encephalitis, a presentation that is not uncommon in children. This study demonstrated that VGKC Abs are potentially an important cause for encephalitis with severe seizures in children. The affected children in our study had features similar to those described in adults with VGKC Ab encephalitis (Vincent et al., 2004), including encephalopathy, temporal lobe seizures and mild CSF abnormalities. However Brain MRI showed non-specific or minimal abnormalities in our patients, unlike adult patients who often have mesial temporal lobe signal abnormalities. The children who were positive for VGKC Abs in our study had poor outcome including ongoing epilepsy and cognitive impairment, although none of them received early immune therapy.

This study was limited by its retrospective nature, the small number of patients, and the lack of acute immune therapy. Future studies need to examine larger cohorts of children with unexplained encephalitis where seizures are an important presenting feature and test them for the different known neuronal Abs, as well as other Abs that may become evident in the future. Studying the role of immunotherapy in these children and its effect on their neurological outcome is also important. A separate encephalitis study is being conducted at the same time at this hospital aiming to study children with different types of encephalitis, and test them for neuronal Abs.

### **5.2 New onset seizures**

The second study (see Chapter 3) aim was to test a large cohort of children with new onset seizures for neuronal antibodies to examine their potential role in paediatric epilepsy. The patients' sera were

examined within six months of seizure onset (within two months in 75% of the cohort). We believe this is a relative strength of the study, as antibody testing in chronic epilepsy patients could possibly be associated with secondary immune abnormalities (i.e. epiphenomena). The study applied the latest version of the ILAE classification (Berg et al., 2010) at a time where the ILAE classification is evolving, which we consider a relative strength of this study. 9.7 % of tested children were positive for neuronal Abs including VGKC, CASPR 2 and NMDAR Abs. This study is the first to describe serum CASPR2 Abs in children with epilepsy. Seven out of the 11 antibody-positive patients (64%) had focal epilepsy of unknown cause and none of the positive patients had encephalitis. The results of this study suggest that neuronal antibodies are present beyond the spectrum of encephalitis and may be an important cause of focal epilepsy of unknown cause in children. Focal seizures are also typical in adults with neuronal Abs (Quek et al., 2012). Many of the affected antibody-positive children in our study had ongoing epilepsy, although none of them received immune therapy.

While this study is important and novel there are many limitations. The new onset seizure study tested a heterogeneous group of patients presenting to a tertiary hospital. The patients had a wide spectrum of seizures and epilepsies, including some with structural and metabolic causes. The yield of this antibody study might have been higher if the patients were selected on the basis of suspicion of autoimmune seizures (see Chapter 4), or if the patients with a known cause for their epilepsies were excluded.

As this study found that neuronal Abs were more common in patients with focal epilepsy of unknown cause, we suggest that future studies target patients with focal epilepsy. Positive neuronal Abs were also found in some patients with a known structural or metabolic causes, as well as in some controls. The significance of this finding is not clear and studying larger cohorts of children with epilepsy and healthy controls are needed to help understand the specificity of neuronal Abs in these cases. It is likely that serum antibodies are not 100% specific, and therefore the results of Ab testing need to be interpreted in the clinical context. However as a screening investigation, one might accept a lower specificity for a higher sensitivity so no patient is missed. CSF testing for neuronal Abs might be



more specific than serum testing however this was not performed in our study due to sample limitation.

We found neuronal antibodies in patients with epilepsy of unknown cause, and as these patients now have a "known" cause for their epilepsy, namely immune, we suggest that an "immune" category is added to the current ILAE classification to incorporate these patients.

In this study none of the positive patients received immunotherapy, and future studies need to examine the role of immune therapy and the effect on epilepsy and neurological outcomes in these patients.

### **5.3 Diagnostic guidelines study**

We studied a miscellaneous group of children presenting with seizures that were suspected to be of autoimmune origin, based on the presence of suggestive features such as CNS inflammation and the presence of other autoimmune disorders (Chapter 4). A proportion of these children were positive for neuronal antibodies, however we think that negative antibody testing does not fully exclude an autoimmune cause, as it is likely that there are as-yet-unidentified anti-neuronal antibodies. We proposed guidelines that might help clinicians in the identification and work up of children with suspected autoimmune seizures. We applied the proposed guidelines to the miscellaneous group of patients with seizures of suspected autoimmune origin, to test their usefulness. We think these guidelines are a starting point to approach these children while our understanding of autoimmune epilepsies in children continues to evolve. Evaluation of these guidelines applicability in larger, prospective cohorts will help validate their usefulness.

These guidelines are clinically oriented, whereas a different laboratory based guideline has been recently proposed by Lancaster et al to identify autoimmune encephalopathy (Lancaster and Dalmau, 2012). These laboratory based guidelines used cell based assays as well as rat brain immunohistochemistry and cultures of neurons for serum and CSF antibody binding; however these methods are not readily available for clinicians but are important for research purposes.

Our proposed guidelines for identification of autoimmune seizures are limited by the lack of understanding of the phenotypic spectrum of autoimmune epilepsy in children. The guidelines represent a broad starting point. Future studies examining larger cohort of patients with suspected autoimmune seizures will help improve clinical and treatment specific guidelines.

#### **5.4 Other insights and future directions**

Children with VGKC complex Abs in our studies (in total 12 patients) were negative for LGI1, CASPR2 and contactin2, which were frequently found to be the targets of VGKC-complex Abs in adults. This suggests that VGKC-complex Abs in children are different to adults and might have other antigenic targets. Future directions should aim at identifying VGKC complex antigen(s) involved in antibody binding in children. Although we found CASPR2 Abs in some of our patients with new onset epilepsy (three cases) we have found no LGI1 antibodies in any of our patients, and it is possible that LGI1 Abs are restricted to adults with autoimmune encephalopathy.

Many neuronal Abs are now known to be associated with epilepsy and seizures; however it is likely that many more are yet to be discovered. We think that some of the patients that are currently negative for the known neuronal Abs might be positive for other unidentified Abs, which means there are unidentified patients with autoimmune epilepsy. Future studies should aim at identifying other autoantigens involved in autoimmune epilepsy.

The "pathogenicity" of these neuronal antibodies is an important area that needs further study. While the research so far indicates that some of these antibodies are pathogenic, it is possible that some of these Abs are secondary to cell damage (an epiphenomenon).

Finally this thesis explores a possible epileptogenic cause, namely auto immune. It is hoped that early identification and intervention, in the form of immune therapy could improve outcomes. Early intervention treatment trials in children with suspected autoimmune epilepsy, particularly previously unknown focal epilepsy, are warranted.

## 6. APPENDICES

### Appendix 1 ILAE classification tables

Tables 1 to 4 text is from Engel 2001 (Engel, 2001), Table 5 text is from Berg et al 2010 (Berg et al., 2010).

*Table 1 Proposed diagnostic scheme for people with epileptic seizures and with epilepsy (Engel 2001).*

Epileptic seizures and epilepsy syndromes are to be described and categorized according to a system that uses standardized terminology, and that is sufficiently flexible to take into account the following practical and dynamic aspects of epilepsy diagnosis:

1. Some patients cannot be given a recognized syndromic diagnosis.
2. Seizure types and syndromes change as new information is obtained.
3. Complete and detailed descriptions of ictal phenomenology are not always necessary.
4. Multiple classification schemes can, and should, be designed for specific purposes (e.g., communication and teaching; therapeutic trials; epidemiologic investigations; selection of surgical candidates; basic research; genetic characterizations).

This diagnostic scheme is divided into five parts, or Axes, organized to facilitate a logical clinical approach to the development of hypotheses necessary to determine the diagnostic studies and therapeutic strategies to be undertaken in individual patients:

Axis 1: Ictal phenomenology, from the Glossary of Descriptive Ictal Terminology, can be used to describe ictal events with any degree of detail needed.

Axis 2: Seizure type, from the List of Epileptic Seizures.

Localization within the brain and precipitating stimuli for reflex seizures should be specified when appropriate.

Axis 3: Syndrome, from the List of Epilepsy Syndromes, with the understanding that a syndromic diagnosis may not always be possible.

Axis 4: Etiology, from a Classification of Diseases Frequently Associated with Epileptic Seizures or Epilepsy Syndromes when possible, genetic defects, or specific pathologic substrates for symptomatic focal epilepsies.

Axis 5: Impairment, this optional, but often useful, additional diagnostic parameter can be derived from an impairment.

**Table 2 Definitions of key terms (Engel 2001).**

Epileptic seizure type: An ictal event believed to represent a unique pathophysiologic mechanism and anatomic substrate. This is a diagnostic entity with etiologic, therapeutic, and prognostic implications. (new concept)

Epilepsy syndrome: A complex of signs and symptoms that define a unique epilepsy condition. This must involve more than just the seizure type: thus frontal lobe seizures per se, for instance, do not constitute a syndrome. (changed concept).

Epileptic disease: A pathologic condition with a single specific, well-defined etiology. Thus progressive myoclonus epilepsy is a syndrome, but Unverricht–Lundborg is a disease. (new concept)

Epileptic encephalopathy: A condition in which the epileptiform abnormalities themselves are believed to contribute to the progressive disturbance in cerebral function. (new concept)

Benign epilepsy syndrome: A syndrome characterized by epileptic seizures that are easily treated, or require no treatment, and remit without sequelae. (clarified concept)

Reflex epilepsy syndrome: A syndrome in which all epileptic seizures are precipitated by sensory stimuli. Reflex seizures that occur in focal and generalized epilepsy syndromes that also are associated with spontaneous seizures are listed as seizure types. Isolated reflex seizures also can occur in situations that do not necessarily require a diagnosis of epilepsy. Seizures precipitated by other special circumstances, such as fever or alcohol withdrawal, are not reflex seizures. (changed concept)

Focal seizures and syndromes: Replaces the terms partial seizures and localization-related syndromes. (changed terms)

Simple and complex partial epileptic seizures: These terms are no longer recommended, nor will they be replaced. Ictal impairment of consciousness will be described when appropriate for individual seizures, but will not be used to classify specific seizure types. (new concept)

Idiopathic epilepsy syndrome: A syndrome that is only epilepsy, with no underlying structural brain lesion or other neurologic signs or symptoms. These are presumed to be genetic and are usually age dependent. (unchanged term)

Symptomatic epilepsy syndrome: A syndrome in which the epileptic seizures are the result of one or more identifiable structural lesions of the brain. (unchanged term)

Probably symptomatic epilepsy syndrome: Synonymous with, but preferred to, the term cryptogenic, used to define syndromes that are believed to be symptomatic, but no etiology has been identified. (new term)

**Table 3 Epileptic seizure types and precipitating stimuli for reflex seizures (Engel 2001).**

Self-limited seizure types

Generalized seizures

- Tonic–clonic seizures (includes variations beginning with a clonic or myoclonic phase)
- Clonic seizures
  - Without tonic features
  - With tonic features
- Typical absence seizures
- Atypical absence seizures
- Myoclonic absence seizures
- Tonic seizures
- Spasms
- Myoclonic seizures
- Eyelid myoclonia
  - Without absences
  - With absences
- Myoclonic atonic seizures
- Negative myoclonus
- Atonic seizures
- Reflex seizures in generalized epilepsy syndromes

Focal seizures

Focal sensory seizures

- With elementary sensory symptoms (e.g., occipital and parietal lobe seizures)
- With experiential sensory symptoms (e.g., temporoparietooccipital junction seizures)

Focal motor seizures

- With elementary clonic motor signs
- With asymmetric tonic motor seizures (e.g., supplementary motor seizures)
- With typical (temporal lobe) automatisms (e.g., mesial temporal lobe seizures)
- With hyperkinetic automatisms
- With focal negative myoclonus
- With inhibitory motor seizures

Gelastic seizures

Hemiclonic seizures

Secondarily generalized seizures

Reflex seizures in focal epilepsy syndromes

Continuous seizure types

Generalized status epilepticus

- Generalized tonic–clonic status epilepticus
- Clonic status epilepticus
- Absence status epilepticus
- Tonic status epilepticus
- Myoclonic status epilepticus

Focal status epilepticus

- Epilepsia partialis continua of Kojevnikov
- Aura continua
- Limbic status epilepticus (psychomotor status)
- Hemiconvulsive status with hemiparesis

Precipitating stimuli for reflex seizures

Visual stimuli

- Flickering light: color to be specified when possible

- Patterns
- Other visual stimuli
- Thinking
- Music
- Eating
- Praxis
- Somatosensory
- Proprioceptive
- Reading
- Hot water
- Startle

***Table 4 Epilepsy syndromes and related conditions (Engel 2001).***

- Benign familial neonatal seizures
- Early myoclonic encephalopathy
- Ohtahara syndrome
- Migrating partial seizures of infancy
- West syndrome
- Benign myoclonic epilepsy in infancy
- Benign familial infantile seizures
- Benign infantile seizures (nonfamilial)
- Dravet's syndrome
- HH syndrome
- aMyoclonic status in nonprogressive encephalopathies
- Benign childhood epilepsy with centrotemporal spikes
- Early-onset benign childhood occipital epilepsy (Panayiotopoulos type)
- Late-onset childhood occipital epilepsy (Gastaut type)
- Epilepsy with myoclonic absences
- Epilepsy with myoclonic–astatic seizures
- Lennox–Gastaut syndrome
- Landau–Kleffner syndrome (LKS)
- Epilepsy with continuous spike-and-waves during slow-wave sleep (other than LKS)
- Childhood absence epilepsy
- Progressive myoclonus epilepsies
- Idiopathic generalized epilepsies with variable phenotypes
  - Juvenile absence epilepsy
  - Juvenile myoclonic epilepsy
  - Epilepsy with generalized tonic–clonic seizures only
- Reflex epilepsies
  - Idiopathic photosensitive occipital lobe epilepsy
  - Other visual sensitive epilepsies

- Primary reading epilepsy
- Startle epilepsy
- Autosomal dominant nocturnal frontal lobe epilepsy
- Familial temporal lobe epilepsies
- Generalized epilepsies with febrile seizures plus
- Familial focal epilepsy with variable foci
- Symptomatic (or probably symptomatic) focal epilepsies
  - Limbic epilepsies
    - Mesial temporal lobe epilepsy with hippocampal sclerosis
    - Mesial temporal lobe epilepsy defined by specific etiologies
    - Other types defined by location and etiology
  - Neocortical epilepsies
    - Rasmussen syndrome
    - Other types defined by location and etiology
- Conditions with epileptic seizures that do not require a diagnosis of epilepsy
  - Benign neonatal seizures
  - Febrile seizures
  - Reflex seizures
  - Alcohol-withdrawal seizures
  - Drug or other chemically induced seizures
  - Immediate and early posttraumatic seizures
  - Single seizures or isolated clusters of seizures
  - Rarely repeated seizures (oligoepilepsy)

***Table 5 Electroclinical syndromes and other epilepsies (Berg et al 2010).***

Electroclinical syndromes arranged by age at onset <sup>a</sup>

Neonatal period

Benign familial neonatal epilepsy (BFNE)

Early myoclonic encephalopathy (EME)

Ohtahara syndrome

Infancy

Epilepsy of infancy with migrating focal seizures

West syndrome

Myoclonic epilepsy in infancy (MEI)

Benign infantile epilepsy

Benign familial infantile epilepsy

Dravet syndrome

Myoclonic encephalopathy in nonprogressive disorders

Childhood

- Febrile seizures plus (FS+) (can start in infancy)
- Panayiotopoulos syndrome
- Epilepsy with myoclonic atonic (previously astatic) seizures
- Benign epilepsy with centrotemporal spikes (BECTS)
- Autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE)
- Late onset childhood occipital epilepsy (Gastaut type)
- Epilepsy with myoclonic absences
- Lennox-Gastaut syndrome
- Epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS)<sup>b</sup>
- Landau-Kleffner syndrome (LKS)
- Childhood absence epilepsy (CAE)

Adolescence – Adult

- Juvenile absence epilepsy (JAE)
- Juvenile myoclonic epilepsy (JME)
- Epilepsy with generalized tonic–clonic seizures alone
- Progressive myoclonus epilepsies (PME)
- Autosomal dominant epilepsy with auditory features (ADEF)
- Other familial temporal lobe epilepsies

Less specific age relationship

- Familial focal epilepsy with variable foci (childhood to adult)
- Reflex epilepsies

Distinctive constellations

- Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE with HS)
- Rasmussen syndrome
- Gelastic seizures with hypothalamic hamartoma
- Hemiconvulsion–hemiplegia–epilepsy

Epilepsies that do not fit into any of these diagnostic categories can be distinguished first on the basis of the presence or absence of a known structural or metabolic condition (presumed cause) and then on the basis of the primary mode of seizure onset (generalized vs. focal)

Epilepsies attributed to and organized by structural-metabolic causes

- Malformations of cortical development (hemimegalencephaly, heterotopias, etc.)
- Neurocutaneous syndromes (tuberous sclerosis complex, Sturge-Weber, etc.)
- Tumor
- Infection
- Trauma



Angioma

Perinatal insults

Stroke

Etc.

Epilepsies of unknown cause

Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy per se

Benign neonatal seizures (BNS)

Febrile seizures (FS)

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<sup>a</sup> The arrangement of electroclinical syndromes does not reflect etiology.

<sup>b</sup> Sometime referred to as Electrical Status Epilepticus during Slow Sleep (ESES).

**Appendix 2 Glossary of Descriptive Terminology for Ictal Semiology (Blume et al., 2001)**

## ILAE Commission Report

# Glossary of Descriptive Terminology for Ictal Semiology: Report of the ILAE Task Force on Classification and Terminology

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## INTRODUCTION

This glossary intends to provide a standard terminology for health care workers to communicate what is observed and what a patient reports during a seizure. As this terminology is descriptive and phenomenologic, its use would not imply or require knowledge of ictal pathophysiology, any pathological substrate, or etiology.

Many terms are adjectives modifying "seizure," which itself is defined under "general terms." This pertains to seizures with single or multiple components.

Terms in this glossary (e.g., "seizure," "ictus," which have widespread applicability in other fields of clinical neuroscience) are herein defined according to their references to epilepsy.

Some terms of this glossary are "fundamental" (i.e., they encompass other more precise words). These can be used as the sole descriptor when data to characterize a phenomenon more precisely are not available. Such include aura, automatism, experiential, motor, and sensory.

A seizure will often consist of two or more phenomena occurring simultaneously or sequentially and should be described accordingly.

Quantitative terms, such as duration of motor events, are not intended as immutable confines, but as clarifying guides to describe clinically observed events.

Scientific progress dictates an evolution of terms to retain their relevance. However, needs of communication in everyday life require that changes be gradual and evolutionary rather than abrupt and revolutionary. The use of

synonyms in this glossary reflects incidents in which gradual changes are likely.

Terminology in some areas remains unresolved. Therefore we view this glossary as a dynamic process for which feedback will be welcomed.

## PRINCIPLES FOR TERMS AND DEFINITIONS

In developing the "lexique" of this report, we adopted and applied the following principles.

Terms and definitions should

1. Contain features that distinguish *or modify* seizure entities.
2. Be descriptive of the phenomena involved.
3. Comply with terminology of clinical neuroscience.
4. Use current terminology and definitions wherever possible.
5. Contain new terms only if necessary.
6. Be easily translatable to other languages.
7. Be readily understood *and used* by potential users.

## I GENERAL TERMS

### 1.0 SEMIOLOGY

That branch of linguistics concerned with signs and symptoms.

### 2.0 EPILEPTIC SEIZURE

Manifestation(s) of epileptic (excessive and/or hypersynchronous), usually self-limited activity of neurons in the brain.

### 3.0 ICTUS

A sudden neurologic occurrence such as a stroke or an epileptic seizure.

### 4.0 EPILEPSY

- a) Epileptic Disorder: A chronic neurologic condition characterized by recurrent epileptic seizures.
- b) Epilepsies: Those conditions involving chronic recurrent epileptic seizures that can be considered epileptic disorders.

### 5.0 FOCAL (syn. partial)

A seizure whose initial semiology indicates, or is consistent with, initial activation of only part of one cerebral hemisphere.

### 6.0 GENERALIZED (syn. bilateral)

A seizure whose initial semiology indicates, or is consistent with, more than minimal involvement of both cerebral hemispheres.

### 7.0 CONVULSION

Primarily a lay term. Episodes of excessive, abnormal muscle contractions, usually bilateral, which may be sustained or interrupted.

## II TERMS DESCRIBING EPILEPTIC SEIZURE SEMIOLOGY

These are descriptors of seizures unless specified otherwise.

### 1.0 MOTOR

Involves musculature in any form. The motor event could consist of an increase (positive) or decrease (negative) in muscle contraction to produce a movement.

Unless noted, the following terms are adjectives modifying “motor seizure” or “seizure” (e.g., “tonic motor seizure or dystonic seizure”), and whose definitions can usually be understood as prefaced by “refers to . . .”.

### 1.1 ELEMENTARY MOTOR

A single type of contraction of a muscle or group of muscles that is usually stereotyped and not decomposable into phases. (However, see tonic–clonic, an elementary motor sequence).

#### 1.1.1 TONIC

A sustained increase in muscle contraction lasting a few seconds to minutes.

##### 1.1.1.1 EPILEPTIC SPASM (Formerly Infantile Spasm)

Noun: A sudden flexion, extension, or mixed extension–flexion of predominantly proximal and truncal muscles that is usually more sustained than a myoclonic movement but not so sustained as a tonic seizure (i.e., 1 s). Limited forms may occur: grimacing, head nodding. Epileptic spasms frequently occur in clusters.

##### 1.1.1.2 POSTURAL

Adoption of a posture that may be bilaterally symmetric or asymmetric (as in a “fencing posture”).

##### 1.1.1.2.1 VERSIVE

A sustained, forced conjugate ocular, cephalic, and/or truncal rotation or lateral deviation from the midline.

##### 1.1.1.2.2 DYSTONIC

Sustained contractions of both agonist and antagonist muscles producing athetoid or twisting movements, which, when prolonged, may produce abnormal postures.

### 1.1.2 MYOCLONIC (adjective); MYOCLONUS (noun)

Sudden, brief (<100 ms) involuntary single or multiple contraction(s) of muscle(s) or muscle groups of variable topography (axial, proximal limb, distal).

#### 1.1.2.1 NEGATIVE MYOCLONIC

Interruption of tonic muscular activity for <500 ms without evidence of preceding myoclonia.

### 1.1.2.2 CLONIC

Myoclonus that is regularly repetitive, involves the same muscle groups, at a frequency of 2–3 c/s, and is prolonged. Synonym: rhythmic myoclonus.

#### 1.1.2.2.1 JACKSONIAN MARCH

Noun: Traditional term indicating spread of clonic movements through contiguous body parts unilaterally.

### 1.1.3 TONIC–CLONIC

A sequence consisting of a tonic followed by a clonic phase. Variants such as clonic–tonic–clonic may be seen.

#### 1.1.3.1 GENERALIZED TONIC–CLONIC SEIZURE (syn. bilateral tonic–clonic seizure) (Formerly “Grand Mal” Seizure)

Noun: Bilateral symmetric tonic contraction and then bilateral clonic contractions of somatic muscles, usually associated with autonomic phenomena.

### 1.1.4 ATONIC

Sudden loss or diminution of muscle tone without apparent preceding myoclonic or tonic event lasting \$1 to 2 s, involving head, trunk, jaw, or limb musculature.

### 1.1.5 ASTATIC

Loss of erect posture that results from an atonic, myoclonic, or tonic mechanism. Synonym: drop attack.

### 1.1.6 SYNCHRONOUS (Asynchronous)

Motor events occurring (not) at the same time or at the same rate in sets of body parts.

## 1.2 AUTOMATISM

Noun: A more or less coordinated, repetitive, motor activity usually occurring when cognition is impaired and for which the subject is usually amnesic afterward. This often resembles a voluntary movement and may consist of an inappropriate continuation of ongoing preictal motor activity.

The following adjectives are usually employed to modify “automatism.”

### 1.2.1 OROALIMENTARY

Lip smacking, lip pursing, chewing, licking, tooth grinding, or swallowing.

### 1.2.2 MIMETIC

Facial expression suggesting an emotional state, often fear.

### 1.2.3 MANUAL OR PEDAL

1. Indicates principally distal components, bilateral or unilateral.
2. Fumbling, tapping, manipulating movements.

### 1.2.4 GESTURAL

Often unilateral.

1. Fumbling or exploratory movements with the hand, directed toward self or environment.
2. Movements resembling those intended to lend further emotional tone to speech.

### 1.2.5 HYPERKINETIC

1. Involves predominantly proximal limb or axial muscles producing irregular sequential ballistic movements, such as pedaling, pelvic thrusting, thrashing, rocking movements.
2. Increase in rate of ongoing movements or inappropriately rapid performance of a movement.

### 1.2.6 HYPOKINETIC

A decrease in amplitude and/or rate or arrest of ongoing motor activity.

### 1.2.7 DYSPHASIC

Impaired communication involving language without dysfunction of relevant primary motor or sensory pathways, manifested as impaired comprehension, anomia, paraphasic errors, or a combination of these.

### 1.2.8 DYSPRAXIC

Inability to perform learned movements spontaneously or on command or imitation despite intact relevant motor and sensory systems and adequate comprehension and cooperation.

### 1.2.9 GELASTIC

Bursts of laughter or giggling, usually without an appropriate affective tone.

### 1.2.10 DACRYSTIC

Bursts of crying.

### 1.2.11 VOCAL

Single or repetitive utterances consisting of sounds such as grunts or shrieks.

### 1.2.12 VERBAL

Single or repetitive utterances consisting of words, phrases, or brief sentences.

### 1.2.13 SPONTANEOUS

Stereotyped, involve only self, virtually independent of environmental influences.

### 1.2.14 INTERACTIVE

Not stereotyped, involve more than self, environmentally influenced.

## 2.0 NONMOTOR

### 2.1 AURA

Noun: A subjective ictal phenomenon that, in a given patient, may precede an observable seizure; if alone, constitutes a sensory seizure.

## 2.2 SENSORY

A perceptual experience not caused by appropriate stimuli in the external world. Modifies “seizure” or “aura.”

### 2.2.1 ELEMENTARY

A single, unformed phenomenon involving one primary sensory modality (e.g., somatosensory, visual, auditory, olfactory, gustatory, epigastric, or cephalic).

#### 2.2.1.1 SOMATOSENSORY

Tingling, numbness, electric-shock sensation, pain, sense of movement, or desire to move.

#### 2.2.1.2 VISUAL

Flashing or flickering lights, spots, simple patterns, scotomata, or amaurosis.

#### 2.2.1.3 AUDITORY

Buzzing, drumming sounds or single tones.

#### 2.2.1.4 OLFACTORY

Odor, usually disagreeable.

#### 2.2.1.5 GUSTATORY

Taste sensations including acidic, bitter, salty, sweet, or metallic.

#### 2.2.1.6 EPIGASTRIC

Abdominal discomfort including nausea, emptiness, tightness, churning, butterflies, malaise, pain, and hunger; sensation may rise to chest or throat. Some phenomena may reflect ictal autonomic dysfunction.

#### 2.2.1.7 CEPHALIC

Sensation in the head such as light-headedness, tingling or headache.

#### 2.2.1.8 AUTONOMIC

A sensation consistent with involvement of the autonomic nervous system, including cardiovascular, gastrointestinal, sudomotor, vasomotor, and thermoregulatory functions. (Thus “autonomic aura”; cf. “autonomic events” 3.0).

### 2.2.2 EXPERIENTIAL

Affective, mnemonic, or composite perceptual phenomena including illusory or composite hallucinatory events; these may appear alone or in combination. Included are feelings of depersonalization. These phenomena have subjective qualities similar to those experienced in life but are recognized by the subject as occurring outside of actual context.

#### 2.2.2.1 AFFECTIVE

Components include fear, depression, joy, and (rarely) anger.

### 2.2.2.2 MNEMONIC

Components that reflect ictal dysmnnesia such as feelings of familiarity (*déjà-vu*) and unfamiliarity (*jamais-vu*).

### 2.2.2.3 HALLUCINATORY

A creation of composite perceptions without corresponding external stimuli involving visual, auditory, somatosensory, olfactory, and/or gustatory phenomena. Example: “hearing” and “seeing” people talking.

### 2.2.2.4 ILLUSORY

An alteration of actual percepts involving the visual, auditory, somatosensory, olfactory, or gustatory systems.

## 2.3 DYSCOGNITIVE

The term describes events in which (1) disturbance of cognition is the predominant or most apparent feature, and (2a) two or more of the following components are involved, or (2b) involvement of such components remains undetermined. Otherwise, use the more specific term (e.g., “mnemonic experiential seizure” or “hallucinatory experiential seizure”).

Components of cognition:

- perception: symbolic conception of sensory information
- attention: appropriate selection of a principal perception or task
- emotion: appropriate affective significance of a perception
- memory: ability to store and retrieve percepts or concepts
- executive function: anticipation, selection, monitoring of consequences, and initiation of motor activity including praxis, speech

## 3.0 AUTONOMIC EVENTS

### 3.1 AUTONOMIC AURA

A sensation consistent with involvement of the autonomic nervous system, including cardiovascular, gastrointestinal, sudomotor, vasomotor, and thermoregulatory functions (see 2.2.1.8).

### 3.2 AUTONOMIC SEIZURE

An objectively documented and distinct alteration of autonomic nervous system function involving cardiovascular, pupillary, gastrointestinal, sudomotor, vasomotor, and thermoregulatory functions.

## 4.0 SOMATOTOPIC MODIFIERS

### 4.1 LATERALITY

#### 4.1.1 UNILATERAL

Exclusive or virtually exclusive involvement of one side as a motor, sensory, or autonomic phenomenon.

#### 4.1.1.1 HEMI-

A prefix to other descriptors (e.g., hemiclonic).

#### 4.1.2 GENERALIZED (syn. “bilateral”)

More than minimal involvement of each side as a motor, elementary sensory, or autonomic phenomenon.

Motor component: further modified as

##### 4.1.2.1 ASYMMETRIC

Clear distinction in quantity and/or distribution of behavior on the two sides.

##### 4.1.2.2 SYMMETRIC

Virtual bilateral equality in these respects.

## 4.2 BODY PART

Refers to area involved (i.e., arm, leg, face, trunk, and other).

## 4.3 CENTRICITY

Modifier describes proximity to the body axis.

### 4.3.1 AXIAL

Involves trunk, including neck.

### 4.3.2 PROXIMAL LIMB

Signifies involvement from shoulders to wrist, hip to ankle.

### 4.3.3 DISTAL LIMB

Indicates involvement of fingers, hands, toes, and/or feet.

## 5.0 MODIFIERS AND DESCRIPTORS OF SEIZURE TIMING

The following terms are listed in the form (adjective, noun, verb) according to principal usage; as adjective unless specified.

### 5.1 INCIDENCE

Noun: Refers to the number of epileptic seizures within a time period or the number of seizure days per unit of time.

#### 5.1.1 REGULAR, IRREGULAR

Consistent (inconsistent) or predictable (unpredictable, chaotic) intervals between such events.

#### 5.1.2 CLUSTER

1. Noun: Incidence of seizures within a given period (usually one or a few days) that exceeds the average incidence over a longer period for the patient.
2. Verb: To vary in incidence as above.

#### 5.1.3 PROVOCATIVE FACTOR

Noun: Transient and sporadic endogenous or exogenous element capable of augmenting seizure incidence in persons with chronic epilepsy and evoking seizures in susceptible individuals without epilepsy.



### 5.1.3.1 REACTIVE

Occurring in association with transient systemic perturbation such as intercurrent illness, sleep loss, or emotional stress.

### 5.1.3.2 REFLEX

Objectively and consistently demonstrated to be evoked by a specific afferent stimulus or by activity of the patient. Afferent stimuli can be *elementary* [i.e., unstructured (light flashes, startle, a monotone)] or *elaborate* [i.e., structured, (a symphony)]. Activity may be elementary [e.g., motor (a movement)]; or elaborate [e.g., cognitive function (reading, chess playing)], or both (reading aloud).

## 5.2 STATE DEPENDENT

Occurring exclusively or primarily in the various stages of drowsiness, sleep, or arousal.

## 5.3 CATAMENIAL

Seizures occurring principally or exclusively in any one phase of the menstrual cycle.

## 6.0 DURATION

Time between the beginning of initial seizure manifestations, such as the aura, and the cessation of experienced or observed seizure activity. Does not include nonspecific seizure premonitions or postictal states.

## 6.1 STATUS EPILEPTICUS

A seizure that shows no clinical signs of arresting after a duration encompassing the great majority of seizures of that type in most patients *or* recurrent seizures without interictal resumption of baseline central nervous system function.

## 7.0 SEVERITY

A multicomponent assessment of a seizure by observers and the patient.

Components primarily of observer assessment include duration, extent of motor involvement, impairment of cognitive interaction with environment intraictally, maximal number of seizures per unit of time.

Components primarily of patient assessment: extent of injury; emotional, social, and vocational consequences of the attack.

## 8.0 PRODROME

A preictal phenomenon. A subjective or objective clinical alteration (e.g., ill-localized sensation or agitation) that heralds the onset of an epileptic seizure but does not form part of it.

## 9.0 POSTICTAL PHENOMENON

A transient clinical abnormality of central nervous system function that appears or becomes accentuated when clinical signs of the ictus have ended.

## 9.1 LATERALIZING [TODD'S (OR BRAVAIS')] PHENOMENON

Any unilateral postictal dysfunction relating to motor, language, sensory, and/or integrative functions including visual, auditory, or somatosensory neglect phenomena.

## 9.2 NONLATERALIZING PHENOMENON

Impaired cognition, amnesia, psychosis.

### 9.2.1 IMPAIRED COGNITION

Decreased cognitive performance involving one or more of perception, attention, emotion, memory, execution, praxis, speech (cf., *Dyscognitive*, 2.3).

#### 9.2.1.1 ANTEROGRADE AMNESIA

Impaired ability to remember new material.

#### 9.2.1.2 RETROGRADE AMNESIA

Impaired ability to recall previously remembered material.

### 9.2.2 PSYCHOSIS

Misinterpretation of external world in an awake, alert person; involves thought disorder of emotion and socialization.

## DATA SOURCES

Adams RD, Victor M. *Principles of neurology*. 5th ed. New York: McGraw-Hill, 1993.

Aicardi J. *Epilepsy in children: International Review of Child Neurology series*. New York: Raven Press, 1986, 1994.

Benson DF. *The neurology of thinking*. New York: Oxford University Press, 1994: 224–5.

Blume WT, Berkovic S, Dulac O. Search for a better classification of the epilepsies. In: Engel J Jr, Pedley TA, eds. *Epilepsy: a comprehensive textbook*. Vol 1. Philadelphia: Lippincott-Raven Publishers, 1997:779–89.

Delgado-Escueta AV, Serratosa JM, Medina MT. Myoclonic seizures, and progressive myoclonus epilepsy syndromes. In: Wyllie E, ed. *The treatment of epilepsy: principles and practice*. 2nd ed. Baltimore: Williams & Wilkins, 1996:467–83.

Engel J Jr. *Seizures and epilepsy*. Philadelphia: F.A. Davis Company, 1989.

Engel J Jr, Pedley TA. *Epilepsy: a comprehensive textbook*. Vol. 1–3. Philadelphia: Lippincott-Raven Publishers, 1997.

Gastaut H, Broughton R. *Epileptic seizures: clinical and electrographic features, diagnosis and treatment*. Springfield, Ill: Charles C Thomas, 1972.

Gloor P. Consciousness as a neurological concept in epileptology: a critical review. *Epilepsia* 1986;27(suppl 2):S14–26.

Gloor P. *The temporal lobe and limbic system*. New York: Oxford University Press, 1997.

Hopkins AP, Michael WF. Spinal myoclonus. *J Neurol Neurosurg Psychiatry* 1974;37:1112–5.

Luders H, Acharya J, Baumgartner C, et al. Semiological seizure classification. *Epilepsia* 1998;39:1006–13.



Luders H, Acharya J, Baumgartner C, et al. A new seizure classification based exclusively on ictal semiology [Editorial]. *Acta Neurol Scand* 1999;99:137–41.

Moscovitch M. Information processing and the cerebral hemispheres. In: Gazzaniga MS, ed. *Handbook of behavioral neurobiology: neuropsychology*. Vol 2. New York: Plenum, 1979:379–446.

Penfield W, Jasper HH. *Epilepsy and functional anatomy of the human brain*. Boston: Little, Brown, 1954.

Pryse-Phillips W. *Companion to clinical neurology*, Boston: Little, Brown and Company, 1995.

Rowland LP. *Merritt's Textbook of Neurology*. 8th ed. Philadelphia: Lea & Febiger, 1989.

So N. Epileptic auras. In: Wyllie E, ed. *The treatment of epilepsy: principles and practice*. 2nd ed. Baltimore: Williams & Wilkins, 1996: 376–84.

Wolf P. *Epileptic seizures and syndromes*. London: John Libbey, 1994.

Young GB. *Coma and impaired consciousness: a clinical perspective*. New York: McGraw-Hill, 1998.

Young GB, Pigott SE. Neurobiological basis of consciousness. *Arch Neurol* 1999;56:153–7.

### **Some nonmedical texts:**

Sykes JB, ed. *The concise Oxford dictionary*. 7th ed. Oxford, England: Clarendon Press, 1982.

*Dictionnaire Encyclopedique. Petit Larousse Illustré*. Librairie Larousse, Paris, 1973.

*Dictionary of Canadian English: the senior dictionary*. Avis WS, Drysdale PD, Gregg RJ, Scargill MH, eds. Toronto, Canada: W.J. Gage, 1967.

## Appendix 3 Ethics approvals and related forms

### A 3.1 Human Research Ethics Committee (HREC) approval

the  
children's  
hospital at Westmead

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#### Research and Development

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ETG:\DATA\Research\ETHICS\EC\Project correspondence\2009 files\09.CHW.57 Finapp ltr.doc

25<sup>th</sup> August 2009

Dr Russell Dale  
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Dear Dr Dale,

**HREC reference number:** 09/CHW/57  
**You must quote this number for all future correspondence**

**Project title:** Autoimmune channelopathies in paediatric epilepsy

**NSW Sites listed:** The Children's Hospital at Westmead

Thank you for submitting the above project for single ethical and scientific review. This project was first considered by The Children's Hospital Westmead lead HREC at its meeting held on 15 May 2009. This HREC has been accredited by the NSW Department of Health as a lead HREC under the model for single ethical and scientific review.

This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Research Involving Humans* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*.

I am pleased to advise that the Committee has granted ethical approval of this research project. The documents reviewed and approved include:

| Document                           | Version | Date                       |
|------------------------------------|---------|----------------------------|
| Application AB/8624/1              |         | April 2009                 |
| Information Sheet for Parents      | 2       | 10 <sup>th</sup> June 2009 |
| Information Sheet for Young People | 2       | 10 <sup>th</sup> June 2009 |
| Consent Form                       | 2       | 10 <sup>th</sup> June 2009 |

Please note the following conditions of approval:

1. The co-ordinating investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:
  - Unforeseen events that might affect continued ethical acceptability of the project.
2. Proposed changes to the research protocol, conduct of the research, or length of HREC approval, will be provided to the HREC for review in the specified format.

3. The HREC will be notified, giving reasons, if the project is discontinued at a site before the expected date of completion.
4. The co-ordinating investigator will provide an annual report to the HREC and at completion of the study. The annual report form is available on the Hospital's intranet and internet or from the Secretary.
5. Your approval is valid for 5 years from the date of the final approval letter. If your project extends beyond five years – at the 5 year anniversary you are required to resubmit your protocol, according to the latest guidelines, seeking the renewal of your previous approval. In the event of a project **not having commenced** within 12 months of its approval, the approval will lapse and reapplication to the Ethics Committee will be required.

Should you have any queries about the HREC's consideration of your project please contact Ms Eleanor Thackray, Secretary of the Ethics Committee on 9845 3017.

**You are reminded that this letter constitutes ethical approval only. You must not commence this research project at a site until separate authorisation from the Chief Executive or delegate of that site has been obtained.**

A copy of this letter must be forwarded to all site investigators for submission to the relevant Research Governance Officer.

The HREC wishes you every success in your research.

Yours faithfully,



Ms Eleanor Thackray  
Secretary, Ethics Committee

## A 3.2 Site Specific Assessment (SSA) approval

the  
children's  
hospital at Westmead

---

### Research and Development

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27 October 2009

Dr Russell Dale  
Clinical School

Dear Dr Dale,

**HREC reference number:** HREC/09/CHW/57

**SSA reference number:** SSA/09/CHW/142

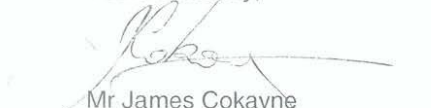
**Project title:** Autoimmune channelopathies in paediatric epilepsy

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place here at The Children's Hospital at Westmead.

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and which are submitted to the lead HREC for review, are copied to the research governance officer;
2. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the research governance officer.

Yours sincerely,



Mr James Cokayne  
Research Governance Officer

## A 3.3 Annual report



The Sydney Children's  
Hospitals Network  
(Randwick and Westmead)

---

**Research and Development**  
**Contact for this correspondence:**

Ms Righa Saroo  
[righa.saroo@health.nsw.gov.au](mailto:righa.saroo@health.nsw.gov.au)  
Phone: (02) 9845 1253  
Facsimile: (02) 9845 1317

Date: 3.4.2012

Dr Russell Dale  
Clinical School  
CHW

Dear Dr Dale,

**Project Title:** Autoimmune channelopathies in paediatric epilepsy  
**Project Number:** 09/CHW/57  
**Status of Project:** Ongoing / In Progress

**Original Ethics Approval:** 25 August 2009  
**Next Annual Review Due:** 25 August 2012  
(Annual reviews are due on the anniversary of ethics approval)  
**Expiry of Ethics Approval:** 25 August 2014

Thank you for your annual report received on 27 January 2012 for the above study. This report was noted at the Sydney Children's Hospitals Network Human Research Ethics Committee (HREC) meeting on 16 February 2012.

The General Conditions of ethics approval have been satisfied and your approval is valid. We wish you continued success with your project.

Please do not hesitate to contact us should you have any queries.

Yours sincerely,

Signature: 

Date: 3.4.2012

Dr Peter Cooper  
**Chair,**  
**Sydney Children's Hospitals Network Human Research Ethics Committee**

CC: Dr Jehan Suleiman, Neurology, CHW

G:\DATA\Research\ETHICS\EC\Project Files\NEAF\2009\09CHW57\Correspondence & emails\09CHW57\_Annual report acknowledgement\_2012.doc

## A 3.4 Information sheets and consent forms

the  
children's  
hospital at Westmead

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### INFORMATION SHEET FOR PARENTS

#### **Autoimmune channelopathies in childhood epilepsy.**

##### *Primary Investigator*

Dr Jehan Suleiman, Research fellow, Neurology Department, The Children's Hospital at Westmead

Phone Number: 02 98453404

##### *Other Investigators*

Dr Russell Dale, Consultant neurologist, Neurology Department, The Children's Hospital at Westmead ,

Phone Number: 02 9845 3404

Dr Deepak Gill, Consultant neurologist, Neurology Department, The Children's Hospital at Westmead

Phone Number: 02 98450000

We would like you to consider allowing your child to participate in a research study that will be conducted in the Neurology Department at The Children's Hospital at Westmead.

#### **What is the study about?**

Our study is about investigating what causes epilepsy in young children. Some children with epilepsy have a known cause for their epilepsy; however in most cases the cause is unknown.

Some children's epilepsy is due to genetic inheritance. Another possibility is that the body's immune system affects the way the brain works, resulting in seizures. This study will determine whether patients with epilepsy have antibodies in the blood that affect the way brain cells work.

We hope that a better understanding of what causes epilepsy will lead to better treatment. For example, if patients with epilepsy have antibodies that affect brain cell function, patients may benefit from treatments to reduce this immune reaction.

#### **Who can participate?**



Children who have epilepsy (defined as having had more than 2 seizures at any time in their life) between 1-18 years of age can participate in this study.

**What will the study involve?**

Your child will have blood tests as part of your child's routine investigation into the cause and treatment of seizures. This study does not require any additional needles or blood tests, but we ask that an extra 1 ml of blood is taken at the time of blood collection for use in the research study. This extra blood will be used to measure specific antibodies that affect brain cells. The blood will be stored in the research laboratory until all antibody tests have been performed- once complete the blood will then be discarded.

This study will also involve us reviewing your child's medical records to get information about the seizures and type of epilepsy. If this information cannot be retrieved from the medical records, we would like permission to contact you by phone for more information. Note that this will take 10-15 minutes of your time if required.

**Are there any benefits for you participating in the study?**

There are no known benefits for your child participating in this study. At this time, these results will not affect routine care for children with epilepsy. We hope that the results from this study will improve our understanding of epilepsy and its causes and potentially create new treatments in the future.

**Are there any side-effects and risk associated with this study?**

The blood tests are required as a routine part of your child's management. The blood tests are safe procedures performed frequently, but cause some discomfort. Blood tests can be uncomfortable and there may be some bruising afterwards. We can use a local anaesthetic cream on your child's skin if he/she wishes to do so to reduce the pain.

**Other information**

The information we collect about your child for this study will be confidential. All data will be stored on a password secured computer, accessed only by the doctors involved in this study. The data will be stored for the duration of the study (4 years) and will be deleted once the study has been completed.

Participation in this project is voluntary and if you decide not to take part or decide to withdraw at any time this will not otherwise affect your child's care at the Hospital.

If you have any questions about this study, please do not hesitate to discuss them with the investigators whose details are at the beginning of this information sheet.

Although results from this study will not be available for a number of years, the results can be made available to you in the future if you wish.

**This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact Eleanor Thackray, Secretary of the Ethics Committee (02 9845 3017).**

This Information Sheet is for you to keep. We will also give you a copy of the signed consent form.



## INFORMATION SHEET FOR YOUNG PEOPLE

### **Autoimmune channelopathies in childhood epilepsy.**

#### *Primary Investigator*

Dr Jehan Suleiman, Research fellow, Neurology Department, The Children's Hospital at Westmead  
Phone Number: 02 98453404

#### *Other Investigators*

Dr Russell Dale, Consultant neurologist, Neurology Department, The Children's Hospital at Westmead ,  
Phone Number: 02 9845 3404

Dr Deepak Gill, Consultant neurologist, Neurology Department, The Children's Hospital at Westmead  
Phone Number: 02 98450000

We would like you to consider participating in a research study that will be conducted in the Neurology Department at The Children's Hospital at Westmead.

#### **What is the study about?**

Our study is about investigating what causes epilepsy in young people. Some people with epilepsy have a known cause for their epilepsy; however in most cases the cause is unknown.

Some people's epilepsy is due to genetic inheritance. Another possibility is that the body's immune system affects the way the brain works, resulting in seizures. This study will determine whether patients with epilepsy have antibodies in the blood that affect the way brain cells work.

We hope that a better understanding of what causes epilepsy will lead to better treatment. For example, if patients with epilepsy have antibodies that affect brain cell function, patients may benefit from treatments to reduce this immune reaction.

#### **Who can participate?**

People who have epilepsy (defined as having had more than 2 seizures at any time in their life) between 1-18 years of age can participate in this study.

**What will the study involve?**

You will have blood tests as part of your routine investigation into the cause and treatment of seizures. This study does not require any additional needles or blood tests, but we ask that an extra 1 ml of blood is taken at the time of blood collection for use in the research study. This extra blood will be used to measure specific antibodies that affect brain cells. The blood will be stored in the research laboratory until all antibody tests have been performed- once complete the blood will then be discarded.

This study will also involve us reviewing your medical records to get information about the seizures and type of epilepsy. If this information cannot be retrieved from the medical records, we would like permission to contact you by phone for more information. Note that this will take 10-15 minutes of your time if required.

**Are there any benefits for you participating in the study?**

There are no known benefits for you participating in this study. At this time, these results will not affect routine care for people with epilepsy. We hope that the results from this study will improve our understanding of epilepsy and its causes and potentially create new treatments in the future.

**Are there any side-effects and risk associated with this study?**

The blood tests are required as a routine part of your management. The blood tests are safe procedures performed frequently, but cause some discomfort. Blood tests can be uncomfortable and there may be some bruising afterwards. We can use a local anaesthetic cream on your skin if you wish to do so to reduce the pain.

**Other information**

The information we collect about you for this study will be confidential. All data will be stored on a password secured computer, accessed only by the doctors involved in this study. The data will be stored for the duration of the study (4 years) and will be deleted once the study has been completed.

Participation in this project is voluntary and if you decide not to take part or decide to withdraw at any time this will not otherwise affect your care at the Hospital.

If you have any questions about this study, please do not hesitate to discuss them with the investigators whose details are at the beginning of this information sheet.

Although results from this study will not be available for a number of years, the results can be made available to you in the future if you wish.

**This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact Eleanor Thackray, Secretary of the Ethics Committee (02 9845 3017).**

This Information Sheet is for you to keep. We will also give you a copy of the signed consent form.

**CONSENT FORM**

**Autoimmune channelopathies in childhood epilepsy.**

*Primary Investigator*

Dr Jehan Suleiman, Research fellow, Neurology Department, The Children's Hospital at Westmead

Phone Number: 02 98453404

*Other Investigators*

Dr Russell Dale, Consultant neurologist, Neurology Department, The Children's Hospital at Westmead ,

Phone Number: 02 9845 3404

Dr Deepak Gill, Consultant neurologist, Neurology Department, Children Hospital at Westmead

Phone Number:02 9845 0000

I have read and understand the Information Sheet, and give my consent for my child to participate in this research study, which has been explained to me by

\_\_\_\_\_

I understand that my child is free to withdraw from the study at any time and this decision will not otherwise affect my child's treatment at the Hospital.

NAME OF CHILD: \_\_\_\_\_ (Please print)

SIGNATURE OF CHILD: \_\_\_\_\_ Date: \_\_\_\_\_

NAME OF PARENT OR GUARDIAN: \_\_\_\_\_ (Please print)

SIGNATURE OF PARENT OR GUARDIAN: \_\_\_\_\_ Date: \_\_\_\_\_

NAME OF WITNESS: \_\_\_\_\_ (Please print)

SIGNATURE OF WITNESS: \_\_\_\_\_ Date: \_\_\_\_\_

NAME OF INTERPRETER: \_\_\_\_\_ (Please print)

SIGNATURE OF INTERPRETER: \_\_\_\_\_ Date: \_\_\_\_\_

**Appendix 4 Data base collected for encephalitis and status epilepticus study (Chapter 2)**

|                             |   |  |
|-----------------------------|---|--|
| <b>Patients demographic</b> | Name<br>Medical record number (MRN)<br>Sex<br>Date of birth (DOB)<br>Date of presentation<br>Age at presentation<br>Ethnicity (if known)<br>Paediatrician<br>Neurologist<br>Transfer from other hospital  |  |
| <b>Sample</b>               | Sample date<br>Sample accession number<br>Sample timing in relation to presentation (days)  |  |
| <b>Past medical history</b> | Previously normal<br>Preceding epilepsy<br>Preceding intractable epilepsy<br>Previous status epilepticus<br>Previous febrile seizures<br>Learning difficulty/ developmental delay<br>Behavioural/psychiatric disorder<br>Motor deficit<br>Other neurologic diagnosis<br>Other medical diagnosis | Yes or no  |
| <b>Family history</b>       | Of epilepsy in first degree relatives   |  |
| <b>Status epilepticus</b>   | Status type<br>Status type<br>Clusters or prolonged seizures<br>Febrile or afebrile<br>Duration of status<br>Status treatment   | Convulsive or non convulsive<br>Focal or generalised |

|                                       |  |  |
|---------------------------------------|--|--|
| <b>Hospital admission</b>             | Length of stay (days)<br>PICU admission<br>Length of PICU admission (days)   | Yes or no  |
| <b>Features of presenting illness</b> | Encephalopathy<br>Movement disorder<br>Behavioural alteration<br>Cognitive alteration<br>Inter-current infection<br>Recent infection<br>Type of infection<br>CNS infection<br>Vaccination<br>Other |  |
| <b>Investigations</b>                 | EEG  | Slow- focal or generalised<br>Epileptic- focal or generalised<br>EEG seizure- yes or no  |
|                                       | MRI  | Normal or abnormal<br>MRI details if abnormal  |
|                                       | CSF  | Cells (value)<br>Protein (value)<br>Neopterin(value)<br>OCB (yes or no)<br>PCR for HSV and enterovirus<br>-if done (positive or negative)                            |
|                                       | Serology- if done  | mycoplasma pneumoniae ,<br>enterovirus, cytomegalovirus ,<br>Epstein-Barr virus , herpes<br>simplex virus , human<br>herpesvirus 6 , influenza , and<br>adenovirus . |
|                                       | Other relevant investigations  | Serum sodium, ANA, other<br>autoimmune screening   |
| <b>Immunotherapy</b>                  | Steroids<br>IVIG<br>Other  |  |

|                |                                     |                      |
|----------------|-------------------------------------|----------------------|
| <b>Outcome</b> | Length of follow up (months)        |                      |
|                | Isolated event                      | Yes or no            |
|                | New deficit                         | Yes or no            |
|                | Motor or focal deficit              |                      |
|                | Cognitive difficulties              |                      |
|                | Behavioural /psychiatric impairment |                      |
|                | Other deficits                      |                      |
|                | Ongoing epilepsy                    |                      |
|                | Intractable epilepsy                |                      |
|                | Epilepsy type                       | Focal or generalised |
|                | ILAE classification on follow up    |                      |
|                | Death                               | Yes or no            |



## Appendix 5: Data base collected for new onset seizures study (Chapter 3)

|                             |  |  |
|-----------------------------|--|--|
| <b>Patients demographic</b> | Name<br>MRN<br>Sex<br>DOB<br>Date of onset<br>Age at onset<br>Ethnicity (if known)<br>Paediatrician<br>Neurologist<br>Transfer from other hospital                             |  |
| <b>Sample</b>               | Sample date<br>Sample accession number<br>Sample timing in relation to presentation (days)   |  |
| <b>Past medical history</b> | Previously normal<br>Learning difficulty/<br>developmental delay<br>Behavioural/psychiatric disorder<br>Motor deficit<br>Other neurologic diagnosis<br>Other medical diagnosis | Yes or no                                |
| <b>Family history</b>       | Family history of epilepsy   | In first degree relatives                |
| <b>Presenting seizure</b>   | seizure type<br><br>Febrile or afebrile<br>Clusters<br>Status  | Focal, focal dyscognitive or generalised |
| <b>Hospitalisation</b>      | Hospital admission<br>Length of stay (days)<br>PICU admission<br>Length of PICU admission (days)   | Yes or no<br><br>Yes or no               |

|                                 |   |   |
|---------------------------------|---|---|
| <b>Features on presentation</b> | <p>Encephalopathy</p> <p>Movement disorder</p> <p>Behavioural alteration</p> <p>Cognitive alteration</p> <p>Motor impairment</p> <p>Inter-current illness</p> <p>Type of illness</p> <p>Vaccination</p> <p>Other symptoms or features</p> |   |
| <b>Investigations</b>           | EEG   | <p>Background abnormality</p> <p>Slow- focal or generalised</p> <p>Epileptic- focal or generalised</p> <p>EEG seizure- yes or no</p> <p>EEG diagnosis</p>   |
|                                 | MRI   | <p>Normal or abnormal</p> <p>MRI details</p> <p>MRI diagnosis</p>   |
|                                 | CSF   | <p>Cells (value)</p> <p>Glucose (value)</p> <p>Protein (value)</p> <p>Neopterin(value)</p> <p>OCB (positive/negative/not done)</p> <p>PCR for HSV and enterovirus -if done (positive or negative)</p> |
|                                 | Serology- if done   | <p>mycoplasma pneumoniae ,</p> <p>enterovirus, cytomegalovirus ,</p> <p>Epstein-Barr virus , herpes simplex virus , human herpesvirus 6 , influenza , and adenovirus ,</p> <p>others</p>              |
|                                 | Other relevant investigations   | <p>Serum sodium, ANA, other autoimmune screening, nuclear medicine scans genetic tests etc</p>  |

|  |   |   |
|--|---|---|
| <b>Treatment</b>   | AEDs<br>Steroids<br>IVIG<br>Response to steroids or IVIG<br>Other immunotherapy   | Acute and long term<br><br>Positive or negative |
| <b>Outcome</b><br><br><b>Epilepsy outcome</b><br><br><br><br><br><br><br><br><br><b>Other outcomes</b> | Length of follow up (months)<br>Isolated event<br>New deficit<br>Ongoing epilepsy<br>Intractable epilepsy<br>Number of AED<br>Seizure type on follow up<br>Motor or focal deficit<br>Developmental delay<br>Cognitive difficulties<br>Behavioural /psychiatric impairment | Yes or no                                       |
| <b>Epilepsy classification</b>   | ILAE classification<br>(electroclinical syndromes and others)<br>Aetiology classification (ILAE)  |   |
| <b>Other diagnosis</b>   |   |   |

## **Appendix 6: Publications arising from this work**

# Autoantibodies to neuronal antigens in children with new-onset seizures classified according to the revised ILAE organization of seizures and epilepsies

\*†<sup>1</sup>Jehan Suleiman, ‡<sup>1</sup>Sukhvir Wright, †Deepak Gill, \*Fabienne Brilot, ‡Patrick Waters, §Ken Peacock, †Peter Procopis, ‡Anjan Nibber, ‡Angela Vincent, \*†Russell C. Dale, and ‡Bethan Lang

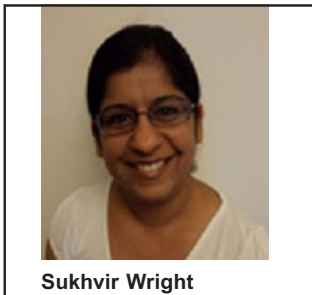
*Epilepsia*, \*\*(\*):1–10, 2013

doi: 10.1111/epi.12405

## SUMMARY



Jehan Suleiman



Sukhvir Wright

**Purpose:** Potentially pathogenic autoantibodies are found increasingly in adults with seizure disorders, including focal seizures and those of unknown cause. In this study, we investigated a cohort of children with new-onset seizures to see whether there were autoantibodies and the relationship to any specific seizure or epilepsy type.

**Methods:** We prospectively recruited 114 children (2 months to 16 years) with new-onset seizures presenting between September 2009 and November 2011, as well as 65 controls. Patients were clinically assessed and classified according to the new International League Against Epilepsy (ILAE) organization of seizures and epilepsies classification system. Sera were tested for autoantibodies to a range of antigens, blind to the clinical and classification details.

**Key Findings:** Eleven (9.7%) of 114 patients were positive for one or more autoantibodies compared to 3 of 65 controls (4.6%,  $p = ns$ ). Patients had antibodies to the voltage-gated potassium channel (VGKC) complex ( $n = 4$ ), contactin-associated protein-like 2 (CASPR2) ( $n = 3$ ), N-methyl-D-aspartate receptors (NMDARs) ( $n = 2$ ), or VGKC-complex and NMDAR ( $n = 2$ ). None had antibodies to glutamic acid decarboxylase, contactin-2, or to glycine, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA), or  $\gamma$ -aminobutyric acid B receptors. Ten of these 11 patients were classified as having epilepsy according to the new ILAE organization of seizures and epilepsy. Although, there were no significant differences in the demographic and clinical features between antibody-positive and antibody-negative patients, the classification of “unknown cause” was higher in the antibody positive (7/10; 70%) compared with the antibody negative subjects (23/86; 26.7%;  $p = 0.0095$ , Fisher’s exact test). Furthermore, four of these seven patients with epilepsy (57.1%) were classified as having predominantly focal seizures compared with 12 of the 86 antibody-negative patients (13.9%;  $p = 0.015$ ).

**Significance:** Because autoantibodies were more frequent in pediatric patients with new-onset epilepsy of “unknown cause,” often with focal epilepsy features, this group of children may benefit most from autoantibody screening and consideration of immune therapy.

**KEY WORDS:** Paediatrics, Epilepsy, Autoimmune, ILAE, VGKC, N-methyl-D-aspartate receptors, Glutamic acid decarboxylase, CASPR2.

Accepted September 3, 2013.

\*Neuroimmunology Group, Institute for Neuroscience and Muscle Research, The Children’s Hospital at Westmead, University of Sydney, Sydney, New South Wales, Australia; †T.Y. Nelson Department of Neurology, Children’s Hospital at Westmead, University of Sydney, Sydney, New South Wales, Australia; ‡Nuffield Department of Clinical Neurosciences, Level 5/6 West Wing, John Radcliffe Hospital, Oxford, United Kingdom; and §Department of General Medicine, Children’s Hospital at Westmead, Sydney, New South Wales, Australia

<sup>1</sup>Contributed equally to work.

Address correspondence to Bethan Lang, Nuffield Department of Clinical Neurosciences, Level 5/6, West Wing, John Radcliffe Hospital, Oxford, OX3 9DS, U.K. E-mail: bethan.lang@ndcn.ox.ac.uk

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A clear clinical diagnosis of patients who present with seizures and the subsequent accurate classification of their epilepsies is extremely important to the neurologist for treatment and prognosis and to the neuroscientist for facilitation of focused, robust research. To meet these dual needs, the revised International League Against Epilepsy (ILAE) system for the organization of seizures and epilepsies, published in 2010, presented a conceptualized framework for everyday clinical practice that also tried to reflect the advancement of basic epilepsy research (Berg et al., 2010). The most important and subsequent controversial recommendation, involved the replacement of the terms “idiopathic, symptomatic, and cryptogenic” with “genetic, structural/metabolic, and unknown cause” when describing etiology (Shinnar, 2010; Panayiotopoulos, 2011, 2012).

It is estimated that epilepsies of unknown cause account for approximately one third of all cases of epilepsy in adults (Berg et al., 2010) and 23–35% in children (van Campen et al., 2013). They are an important group for the identification of new etiologies, and an area in which the emerging identification of specific antibodies may be increasingly important. Antibodies (Abs) directed against neuronal surface proteins such as the voltage-gated potassium channel (VGKC) complex and its associated proteins, leucine-rich, glioma inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2) and contactin-2, the *N*-methyl-D-aspartate receptor (NMDAR),  $\gamma$ -aminobutyric acid<sub>B</sub> receptor (GABA<sub>B</sub>R), and 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid receptor (AMPA) and against intracellular proteins such as glutamic acid decarboxylase (GAD) have already been described. The association between these antibodies and seizures is manifest in patients with limbic encephalitis who often have temporal lobe seizures, and NMDAR-Ab encephalitis in which patients can have focal or generalized seizures. In addition, each of the antibodies has been identified in a proportion of patients with epilepsy but without other encephalitic clinical signs or symptoms, such as cognitive impairment or neuropsychiatric features (Peltola et al., 2000; McKnight et al., 2005; Majoie et al., 2006; Irani et al., 2008; Niehusmann et al., 2009; Barajas et al., 2010; Quek et al., 2012; Brenner et al., 2013), prompting the increased recognition of “autoimmune epilepsy” (Palace & Lang, 2000; Bien & Scheffer, 2011; Irani et al., 2011a; Vincent et al., 2011; Nabbout, 2012; Quek et al., 2012). The identification of a putative autoimmune etiology in some forms of epilepsies suggests that early identification of specific autoantibodies and subsequent treatment with immunotherapy could lead to reduced seizure frequency and may improve outcomes (Irani et al., 2011b; Quek et al., 2012).

In children, NMDAR-Ab encephalitis and VGKC-complex-Ab-associated encephalitis have also been recognized, with seizures being an important presenting symptom (Dale et al., 2009b; Florance et al., 2009; Haberlandt et al., 2011; Suleiman et al., 2011a). Fewer reports

are available regarding the association of neuronal antibodies in defined pediatric epilepsies (e.g., Dhamija et al., 2011; Suleiman et al., 2011b, 2013). Here, we recruited a prospective cohort of children with new-onset seizures, recorded their clinical features, and classified them according to the revised ILAE organization of seizures and epilepsies. At the end of the study period, and blinded to the clinical and classification details, the sera taken at admission were tested for autoantibodies.

## METHODS

### Recruitment criteria of patients with new-onset seizures

We prospectively recruited children aged 2 months to 16 years with new-onset seizures presenting to the Children’s Hospital at Westmead Hospital, Sydney between September 2009 and November 2011. One hundred and fourteen children were included in the study (58 females, mean age 4.39 years, median 2.55 years, range 0.13–15.25 years). All patients with a well-described paroxysmal episode compatible with a clinical seizure and who had serum collected within 6 months of seizure onset were included (in and outpatients). Neonates (0–60 days), and patients with clear etiologies for their seizures such as brain tumor, bacterial meningitis, and brain injury (traumatic or ischemic) were excluded from the study.

### Control group

The control group for antibody testing consisted of hospital patients ( $n = 65$ , 27 female, mean age 9 years, median 9.14 years, range 1–16 years) who had serum collected as part of their routine investigations during 2007. The underlying medical problems were immunologic/inflammatory or allergic ( $n = 28$ ); hematologic/oncologic ( $n = 20$ ); or other medical condition such as respiratory, gastrointestinal, and endocrine ( $n = 17$ ). Recruitment of the patients and control groups for this study was approved by the hospital ethics committee, and written consent was obtained from the patients or their families.

### Antibody assays

Patients were tested for antibodies to VGKC complex and NMDAR ( $n = 114$ ); LGI1, CASPR2, and GAD ( $n = 113$ ); glycine receptor (GlyR), AMPAR, contactin-2 ( $n = 112$ ); and GABA<sub>B</sub>R ( $n = 92$ ). The antibodies to VGKC complex and GAD were tested by radioimmunoassays as described previously (McKnight et al., 2005). VGKC-complex-Abs and GAD levels  $>100$  pM and  $100 >U/ml$ , respectively, were considered positive. All other neuronal antibodies were tested by cell-based assays (CBAs) (for full descriptions see Irani et al., 2008, 2010a,b; Dale et al., 2009b). The CBAs were scored on a visual scale 0 (no binding), 1 (low but specific binding)-4 (strong binding to all transfected cells) by two independent observers. If positive, samples were titrated by serial dilution, and the final dilution at which the sample

remained positive (score of 1) was given. The 65 controls were tested at the same time as the new-onset seizure patients for the following antibodies: LGI1, CASPR2, contactin-2, NMDAR, AMPAR, GABA<sub>B</sub>R, GAD, and GlyR (n = 65) and VGKC complex (n = 62). Assays were performed and read by SW who was blinded to the clinical data, and re-scored by an additional reader. Incomplete testing was due to insufficient sample available. Cerebrospinal fluid testing could not be performed in this study owing to limited sample availability.

### Epilepsy classification

Classifications of seizure types, epilepsy, electroclinical syndrome, and etiology were performed by the primary investigator, a pediatric neurologist (JS), and were cross-checked by a pediatric neurologist and epileptologist (DG). Both were blinded to the results of the antibody testing at the time of performing the classification. The seizure type at presentation was determined for the 114 patients and classified where possible using the new ILAE organization of seizures and epilepsies (Engel, 2006; Berg et al., 2010) and terminology for ictal semiology (Blume et al., 2001). Classification of a patient's condition into an epilepsy syndrome was made by reviewing the child's age, neurologic and developmental state, seizure semiology (at onset and on follow up), electroencephalography (EEG), imaging, and other relevant investigations findings. The structure of the latest proposed ILAE organization of seizures and epilepsies (Berg et al., 2010) was used for this purpose in addition to previous ILAE classification when required (including ICE 1989, Engel, 2001; Engel, 2006). Patients who had features compatible with one of the electroclinical syndromes described in the new ILAE organization of seizures and epilepsies were classified as such irrespective of the etiology of their epilepsy. For example, patients with epileptic spasms and hypsarrhythmic EEG were classified as West syndrome, despite some of these patients having a known structural or metabolic cause. Patients who did not fit into any electroclinical syndrome were classified (as per the ILAE 2010 recommendation) on the basis of presence of metabolic diseases or structural abnormalities. The term "epilepsy of unknown cause" was used to classify those patients who did not fit into an electroclinical syndrome and who had no structural or metabolic cause found for their epilepsy. Patients with seizures that are traditionally not diagnosed as a form of epilepsy including febrile seizures were kept in a separate group as per Berg (2010). We also added patients with acute symptomatic (provoked) seizures (Beghi et al., 2010; Beleza, 2012) and with single unprovoked seizures to this group.

### Statistics and data analysis

Fisher's exact test was used to compare categorical data and the Mann-WhitneyU test to compare continuous data variables.

## RESULTS

### Demographic, clinical features, investigations, and treatment of total cohort

One hundred and one of the 114 patients in the study were admitted to the hospital during their initial presentation or during the first 6 months after seizure onset (median length of stay 4.5 days, range 1–360 days). Eighteen patients required admission to intensive care. Thirty-six patients had one or more preexisting developmental, motor, or psychiatric abnormalities including 33 with developmental delay and/or learning difficulty, 14 with motor deficits, and 4 with behavioral or psychiatric disturbance. There was a positive family history of seizures or epilepsy (including febrile seizures) in the first-degree relatives of 19 patients (16.7%).

Nearly half of the patients (n = 56) presented with focal seizures (Table S1) including 12 with focal dyscognitive seizures. Twenty-one patients presented in status epilepticus and 77 patients had an early recurrence of seizures (occurring within 48 h of first seizure onset). At the time of presentation, 32 patients had intercurrent infections including 21 with fever (defined as 37.8°C or higher [axillary temperature]), 7 of whom fulfilled the definition of febrile seizures (Berg et al., 1992). Seizures were associated with other features in 48 patients including encephalopathy (n = 33), behavioral or psychiatric alteration (n = 27), motor impairment (n = 17), cognitive alteration (n = 12), and movement disorder (n = 5).

EEG findings were abnormal in 86 (76.1%) of 113 patients, and contributed significantly to the epilepsy diagnosis and classification in 22 patients (19.5%). Brain magnetic resonance imaging (MRI) scans were abnormal in 52 of 105 patients and were diagnostic or suggestive of a cause of the epilepsy in 30 patients (28.6%). CSF analysis for pleocytosis, protein, neopterin, or infections was performed in 66 (57.9%) of 114 patients and contributed to epilepsy diagnosis in 13 patients (19.7%). Other investigations were performed as clinically indicated including metabolic, genetic, infectious, and immunologic investigations, and contributed to the epilepsy diagnosis in six patients.

Forty-six patients received acute seizure treatment and 85 patients received long-term (7 days or longer) antiepileptic drugs (AEDs). Twenty-three patients received variable regimens of immunotherapy; including steroids alone (oral prednisolone, intramuscular synthetic adrenocorticotrophic hormone [ACTH] and intravenous methyl prednisolone) in 18 and steroids and intravenous immunoglobulins (IVIGs) in 5. In all cases the decision to treat with immunotherapy was made on the basis of a clinical presentation suggestive of an immune-mediated cause (e.g., Rasmussen encephalitis); a recognized immunotherapy responsive seizure syndrome (e.g., West syndrome); or as adjunctive treatment in intractable epilepsy, prior to and independent of autoantibody test results. A positive clinical response, seen in 15 of 23, was defined as clinical improvement in seizures and/or



encephalopathy as judged by the treating clinician. Thirteen of 18 patients given steroids alone showed improvement, including West syndrome (n = 5), epilepsy of unknown cause (n = 4), Lennox-Gastaut syndrome (n = 2), epilepsy attributed to malformation of cortical development (n = 1), and epilepsy attributed to perinatal insult, encephalomalacia (n = 1). Two of five patients who received steroids in combination with IVIG also showed a positive clinical response (Rasmussen encephalitis, n = 1; and basal ganglia encephalitis, n = 1).

Ten (8.8%) of the 114 patients had no follow-up information available; for the remaining 104 patients, the mean length of follow-up (from seizure onset) was 11.7 months (median 11 months, range 1–36 months) at the time of assessment. Eighty-four patients had ongoing epilepsy, including 23 with drug-resistant (refractory) epilepsy (Kwan et al., 2010). Thirty-two patients had new long-term deficits (other than epilepsy), including one or more of the following: developmental delay or cognitive impairment (n = 24), behavioral/psychiatric impairment (n = 18), and motor deficits (n = 13).

### Epilepsy subgroups separated by classification

The 114 patients with new-onset seizures were classified according to the structure of the latest proposed ILAE organization of seizures and epilepsies (Berg et al., 2010). Eighteen patients had seizures not traditionally diagnosed as a form of epilepsy; the remaining 96 patients had epilepsies: 30 with electroclinical syndromes, 33 with epilepsy attributed to structural-metabolic causes, and 33 with epilepsy of unknown cause (Table 1).

### Antibodies and clinical features of positive patients and controls

Eleven (9.7%) of 114 patients with new-onset seizures were positive for one or more of the tested antibodies: VGKC-complex (n = 4), CASPR2 (n = 3), NMDAR (n = 2), and VGKC-complex and NMDAR (n = 2) (Fig. 1A). None of the patients or controls were positive for GAD, GlyR, AMPAR, GABA<sub>B</sub>R, LGI1, or contactin-2 antibodies.

All antibody-positive patients had been admitted to hospital on presentation, compared to 87.4% of the antibody-negative group. The mean duration of hospital stay for the positive patients was 3.72 days (median 3 days, range 2–11 days), but none required admission to the intensive care unit. Two of the antibody-positive patients had pre-existing developmental delay, which was severe in one. There was a positive family history of epilepsy in two patients. One of the patients (case 8) had status epilepticus of 45 min duration at presentation. Early seizure recurrence in the first 48 h of presentation occurred in 7 of the 11 patients. Features associated with seizures on presentation are presented in Table 2. Overall there were no significant differences in the demographic and clinical features between antibody-

**Table 1. Epilepsy classification as for the new ILAE organization of seizures and epilepsies (Berg et al., 2010)**

| Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy (n = 18) | No. of patients |
|--|-----------------|
| Febrile seizures   | 7               |
| Acute symptomatic (provoked seizures)  |                 |
| Infection mediated   | 7 <sup>a</sup>  |
| Posterior reversible encephalopathy syndrome (PRES) secondary to hypertension                          | 1               |
| Asthma   | 1               |
| Single unprovoked seizure  | 2               |
| ILAE classification/electroclinical syndrome (n = 96)  | No. of patients |
| <i>Electroclinical syndrome (n = 30)<sup>b</sup></i>   |                 |
| Infancy  |                 |
| West syndrome  | 9 <sup>c</sup>  |
| Benign infantile epilepsy  | 3               |
| Dravet syndrome  | 3               |
| Myoclonic epilepsy in infancy (MEI)  | 1               |
| Childhood  |                 |
| Lennox Gastaut syndrome (LGS)  | 4 <sup>d</sup>  |
| Febrile seizures plus (FS+)  | 2               |
| Panayiotopoulos syndrome   | 2               |
| Epilepsy with myoclonic-atic seizures  | 2               |
| Benign epilepsy with centrotemporal spikes (BECTS)   | 1               |
| Childhood absence epilepsy (CAE)   | 1               |
| Late-onset childhood occipital epilepsy (Gastaut type)   | 1               |
| Adolescence  |                 |
| Autosomal dominant epilepsy with auditory features (ADEAF)   | 1               |
| <i>Epilepsy attributed to structural-metabolic causes (n = 33)</i>                                     |                 |
| Infections/inflammation  |                 |
| Encephalitis   | 11 <sup>e</sup> |
| Rasmussen syndrome   | 1               |
| Malformation of cortical development (MCD)   | 10              |
| Perinatal insults  | 5               |
| Metabolic  | 3 <sup>f</sup>  |
| Neurocutaneous syndrome  | 3               |
| <i>Epilepsies of unknown cause (n = 33)</i>  |                 |
| Focal  | 16              |
| Generalized  | 10              |
| Spasms   | 4               |
| Undetermined   | 3               |

<sup>a</sup>These patients had a systemic infection that was presumed to have provoked the seizure, but no evidence of CNS infection or inflammation, and either had no fever or were outside the age definition for febrile seizures. The precipitating infections were gastroenteritis (n = 3), upper respiratory tract infection (n = 2), H1N1 influenza (n = 1), fever alone (n = 1).

<sup>b</sup>"The arrangement of electroclinical syndromes does not reflect etiology" (Berg 2010).

<sup>c</sup>3 genetic, 2 metabolic (congenital glycosylation disorder), 1 structural (perinatal insult), 3 unknown.

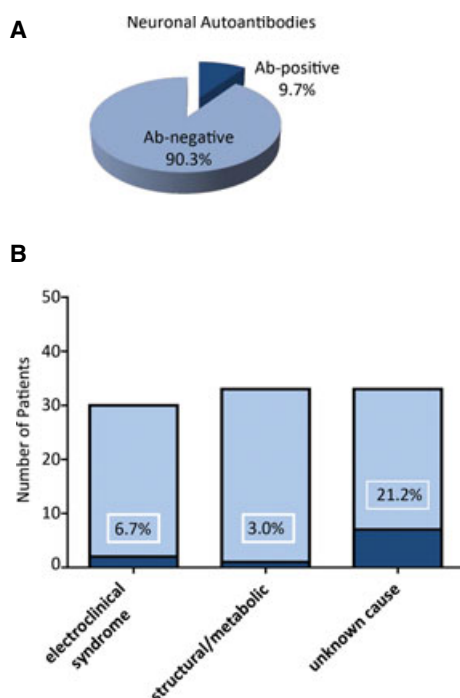
<sup>d</sup>2 structural (malformation of cortical development, holoprosencephaly), 2 unknown.

<sup>e</sup>Encephalitis patients include the following: five with potential infectious etiology, one with dopamine receptor 2 encephalitis, and five not otherwise specified.

<sup>f</sup>MELAS, propionic acidemia, ornithine transcarbamylase deficiency.

positive and antibody-negative patients (Table 3). The timing of the samples from symptom onset for the positive patients was less but not significantly different from that of the antibody-negative patients (mean 17.5; median 4, range





**Figure 1.** ILAE organization of seizures and epilepsies in patients positive for one or more antibodies. **(A)** Antibody positivity (%) in all patients presenting with new-onset seizures,  $n = 114$ . **(B)** ILAE classification of antibody-positive patients with epilepsy ( $n = 10$ ) in relation to the total epilepsy cohort ( $n = 96$ ). Antibody positive, dark blue; antibody negative, light blue. *Epilepsia* © ILAE

1–75 days vs mean 40; median 20 and range 1–180 days, respectively). The mean age was 4.39 years for both positive and negative patients; median was 3.4 years for antibody-positive patients and 2 years for antibody-negative patients.

EEG and MRI brain were performed in 10 of 11 antibody-positive patients. The EEG results were abnormal in 8 and MRI abnormalities were recorded in 4 (Table 2). CSF was tested in only 4 of the 11, and it was normal apart from CSF neopterin, which was elevated in 1 of 3 tested at 68 nM (normal <30; case 1), suggestive of central nervous system (CNS) inflammation (Dale et al., 2009a).

Nine of the positive patients received long-term antiepileptic drugs, but none of the antibody-positive patients received immunotherapy during the period of disease course studied. Two antibody-positive cases were lost to follow-up (cases 8 and nonepileptic case 6). The mean length of follow-up for the remaining nine cases was 10.33 months (median 11 months, range 6–11 months), and was not different from the remaining antibody-negative cases. All of the nine patients with follow-up had ongoing epilepsy, and one had drug-resistant epilepsy (case 9). Five patients were on one AED, three were on two AEDs, and one was on four AEDs. Two patients had a new neurologic or developmental

deficit (other than epilepsy) including speech impairment and hyperactivity (case 3), and motor deficit and behavioral alteration (case 5).

Three of the 65 controls (4.6%) were positive for antibodies. Control 1 (VGKC-complex-Abs 497 pM) was a 3-year-old boy with type 1 diabetes mellitus. Control 2 (VGKC-complex-Abs 200 pM) was a 2-year-old boy with vomiting illness and poor weight gain. Control 3 (NMDAR score 2, end point dilution 1 in 100) was an 8-year-old boy with an acute lymphocytic leukemia relapse treated with bone marrow transplantation, complicated by chronic graft-versus-host disease. No neurologic abnormality was noted in any of these controls, although no natural follow-up was recorded in control 2.

### Patients with antibody-positive new-onset epilepsy according to the new ILAE organization of seizures and epilepsies

One patient with low levels of CASPR2 antibodies had nonepileptic (provoked) seizures (patient 6, Table 2). The remaining 10 antibody-positive patients with epilepsy had the following ILAE epilepsy classifications: electroclinical syndrome ( $n = 2$ ), epilepsy attributed to structural metabolic causes ( $n = 1$ ), and epilepsy of unknown cause ( $n = 7$ ). In the total epilepsy cohort, after exclusion of nonepileptic patients ( $n = 96$ ), only 2 of 30 patients with electroclinical syndromes (6.7%) had positive antibodies (cases 9 and 11). Case 9 had Lennox-Gastaut syndrome (LGS) and antibodies to NMDAR, and case 11 had febrile seizures plus with antibodies to both the NMDAR and VGKC-complex. Similarly, only one of 33 patients with epilepsy attributed to a structural-metabolic cause (3%) had positive antibodies (case 7), again not significantly different from the controls; this case had mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) and was positive for CASPR2 antibodies.

In comparison, 7 of 33 patients from the epilepsy cohort with epilepsy of unknown cause (21.2%) had positive antibodies, which was different from the controls (4.6%;  $p = 0.03$ , Fisher's exact test) (Fig. 1). Overall, the proportion of antibody-positive patients classified with epilepsy of unknown cause (7/10; 70%) was significantly higher than the remaining proportion of antibody-negative epilepsy subjects (23/86; 26.7%;  $p = 0.0095$ , Fisher's exact test) (Fig. 1B). Furthermore, 4 (57%) of these 7 patients had focal epilepsy compared with 12 of the 86 antibody-negative patients (13.9%;  $p = 0.059$ , Table 3).

Of these seven antibody-positive patients with epilepsy of unknown cause, four had VGKC-complex-Abs only (cases 1–4), three of whom presented with focal seizures, and three had early seizure recurrence (cases 1, 3, and 4) (Table 2). Case 5 had CASPR2 antibodies, presented with focal tonic-clonic seizures and had intercurrent infections. Case 8 was positive for NMDAR-Abs and had focal dyscognitive seizures and focal status epilepticus on presentation.

Table 2. Demographic, clinical, electrographic, and imaging features of positive patients (n = 11)

| No.            | Age and sex | Seizure type at onset/<br>early seizure<br>recurrence | Associated<br>features                      | EEG <sup>a</sup>   | MRI   | Seizure type on follow-up                 | Number of<br>AED on<br>follow-up<br>(mo) | ILAE classification                              | Ab positivity<br>titration      |
|----------------|-------------|---|---|--|---|---|--|--|---------------------------------|
| 1              | 2.5 M       | Focal dyscognitive/+                                  | Nil   | Epileptic: left frontotemporal.<br>Seizure: left temporal              | Normal  | Focal dyscognitive, atypical absence      | 1 (11)                                   | Epilepsy of unknown cause (focal-temporal lobe)  | VGKC (293)                      |
| 2              | 4.9 M       | Focal versive/–                                       | Nil   | Epileptic: generalized with bifrontal lead<br>Slowing: right posterior | Normal  | Generalized tonic-clonic                  | 1 (6)                                    | Epilepsy of unknown cause (undetermined)         | VGKC (193)                      |
| 3              | 3.4 M       | Generalized tonic clonic/+                            | Nil   | Normal   | Normal  | Generalized tonic-clonic, atonic          | 2 (6)                                    | Epilepsy of unknown cause (generalized)          | VGKC (182)                      |
| 4              | 1.5 F       | Focal dyscognitive/+                                  | Motor deficit                               | Normal   | Normal  | Focal dyscognitive, atypical absence      | 1 (17)                                   | Epilepsy of unknown cause (focal)                | VGKC (133)                      |
| 5              | 0.5 F       | Focal tonic with secondary generalization/+           | Intercurrent infection                      | Slowing: right temporal<br>Epileptic: central                          | Normal  | Focal tonic with secondary generalization | 1 (11)                                   | Epilepsy of unknown cause (focal)                | CASPR2 (1 in 200 <sup>b</sup> ) |
| 6 <sup>c</sup> | 7 M         | Focal tonic-clonic/–                                  | Intercurrent infection, fever               | Not done   | Not done  | No follow-up                              | –  | Acute symptomatic (provoked) seizure             | CASPR2 (1 in 100)               |
| 7              | 10 M        | Focal myoclonic/+                                     | Encephalopathy, motor deficit (hemiparesis) | Slowing: generalized<br>Epileptic: left parietal                       | Left parietal hyperintensity                    | Focal                                     | 2 (13)                                   | Epilepsy attributed to metabolic cause           | CASPR2 (1 in >400)              |
| 8              | 4.5 M       | Focal dyscognitive/–                                  | Nil   | Slowing: right occipital.  | Normal  | No follow-up                              | –  | Epilepsy of unknown cause (focal-occipital lobe) | NMDAR (1 in 100)                |
| 9              | 3 M         | Generalized myoclonic/+                               | Encephalopathy, behavioral alteration       | Slowing: generalized<br>Epileptic: right frontal                       | Corpus callosum dysgenesis, frontal heterotopia | Mixed (myoclonic, tonic, atonic)          | 4 (12)                                   | Lennox-Gastaut syndrome                          | NMDAR (1 in 100)                |
| 10             | 8 F         | Generalized tonic/–                                   | Preceding infliximab infusion               | High voltage generalized spike and slow wave                           | Generous ventricles                             | Tonic/myoclonic                           | 1 (10)                                   | Epilepsy of unknown cause (generalized)          | VGKC (368) NMDAR (1 in 80)      |
| 11             | 3 F         | Generalized tonic-clonic/+                            | Intercurrent infection                      | Normal   | Asymmetric hippocampi                           | Generalized tonic-clonic                  | 2 (7)                                    | Febrile seizure plus (FS+)                       | VGKC (233) NMDAR (1 in 80)      |

<sup>a</sup>EEG abnormal findings are described in the following order when present: slowing and location, epileptic activity and location, seizure and onset.

<sup>b</sup>CBA were scored on a visual scale: 0 (no binding), 1 (low but specific binding)–4 (strong binding to all transfected cells) by two independent observers. Titration values are highest dilution at which the sample remains positive compared to controls.

<sup>c</sup>Patient defined as nonepileptic with acute symptomatic (provoked) seizure.

**Table 3. Comparison of features of positive and negative cases by ILAE organization of seizures and epilepsies (A) and by clinical characteristics (B)**

| ILAE classification   | Presence of characteristic in Ab-positive patients (%) n = 11 | Presence of characteristic in Ab-negative patients (%) n = 103 | p-Value    |
|---|---|--|------------|
| (A) Epileptic seizures not diagnosed as a form of epilepsy (n = 18) | 1 (9.1)   | 17 (16.5)  | ns         |
| Patients with epilepsy  | n = 10  | n = 86   |            |
| Electroclinical syndrome (n = 30)                                   | 2 (20)  | 31 (36)  | ns         |
| Epilepsy attributed to structural-metabolic causes (n = 33)         | 1 (10)  | 32 (37)  | ns         |
| Epilepsy of unknown cause (n = 33)                                  | 7 (70)  | 23 (26.7)  | 0.0095     |
| Epilepsy of unknown cause (focal) (n = 16)                          | 4 (40)  | 12 (13.9)  | ns (0.059) |
| Epilepsy of unknown cause (generalized) (n = 10)                    | 2 (20)  | 5 (5.8)  | ns         |
| Epilepsy of unknown cause (spasms) (n = 4)                          | 0 (0)   | 4 (4.7)  | ns         |
| Epilepsy of unknown cause (undetermined) (n = 3)                    | 1 (10)  | 2 (2.3)  | ns         |
| (B) Clinical characteristic   |   |  |            |
| Past medical history  |   |  |            |
| Previously normal (n = 78)  | 9 (81.8)  | 69 (67.0)  | ns         |
| First seizure   |   |  |            |
| Focal   | 7 (63.6)  | 49 (47.6)  | ns         |
| Generalized   | 4 (36.4)  | 38 (36.9)  | ns         |
| Status epilepticus  | 1 (9.1)   | 20 (19.4)  | ns         |
| Early seizure recurrence  | 7 (63.6)  | 70 (68.0)  | ns         |
| Hospitalization (n = 101)   | 11 (100)  | 90 (87.4)  | ns         |
| Intensive care (n = 18)   | 0 (0)   | 18 (17.5)  | ns         |
| Associated features   |   |  |            |
| Fever   | 1 (9.1)   | 20 (19.4)  | ns         |
| Infection   | 3 (27.3)  | 28 (27.2)  | ns         |
| Encephalopathy  | 2 (18.2)  | 31 (30.1)  | ns         |
| Movement disorder   | 0 (0)   | 5 (4.8)  | ns         |
| Behavioral abnormality  | 1 (9.1)   | 26 (25.2)  | ns         |
| Cognitive abnormality   | 0 (0)   | 12 (11.8)  | ns         |
| Motor deficit   | 2 (18.2)  | 15 (14.6)  | ns         |
| Outcome   |   |  |            |
| Ongoing epilepsy  | 9 (81.8)  | 75 (72.8)  | ns         |
| New deficit   | 2 (18.2)  | 30 (29.1)  | ns         |
| Developmental delay   | 0 (0)   | 16 (15.5)  | ns         |
| Behavioral/psych impairment   | 2 (18.2)  | 16 (15.5)  | ns         |
| Cognitive impairment  | 0 (0)   | 17 (16.5)  | ns         |

ns: not significant, corrected for multiple comparisons.

Case 10 was double positive for NMDAR and VGKC-complex and had a number of comorbidities. She presented with seizures following an infliximab infusion given for her treatment-resistant Crohn's disease. Infliximab is a monoclonal antibody used in the treatment of some autoimmune diseases and previously described to be associated with seizures (Brigo et al., 2011).

## DISCUSSION

The finding of pathologically relevant autoantibodies to neuronal proteins in a small but significant minority of patients with epilepsy (9–13%, McKnight et al., 2005; Majoie et al., 2006; Brenner et al., 2013) is becoming increasingly recognized, but there have been limited studies in children with epilepsy. Herein we studied a large group of children presenting with new-onset seizures to a tertiary children's hospital and related the results to the recently

revised ILAE organization of seizure and epilepsy. The cohort included a heterogeneous group of patients with a wide spectrum of seizure severity and etiology. Overall antibody positivity was detected in 9.6% of the cohort, and surprisingly in 4.6% of the controls. Nevertheless, in patients with epilepsy of "unknown cause," often with focal seizures, antibodies were more frequent than in the other categories of seizures.

Many patients in the cohort were young and complicated, required intensive acute treatment, or had ongoing refractory epilepsy, as they represented an inpatient sample rather than a community outpatient sample.

The cohort description and classification represented a challenging exercise, particularly at a time when the ILAE classification and terminology is still evolving. It is notable that the patients were classified with investigators blinded to the result of antibody testing. Nearly one third of the patients were determined to have an "unknown cause" for

their epilepsy, as also noted in a recent study comparing the new and old systems (van Campen et al., 2013). Of note, 7 of the 10 antibody-positive results were in this epilepsy category. The most frequently found antibody was to the VGKC-complex (4/10). These four patients, however, were negative for the VGKC-complex proteins LGI1, contactin-2, and CASPR2. The lack of an identified complex protein has been reported previously in children (Dhamija et al., 2011; Ilingworth et al., 2011; Suleiman et al., 2011b; ) and contrasts with adults in whom antibodies to LGI1, and less frequently to CASPR2, are often found (Irani et al., 2008, 2010a,b; Lai et al., 2010), although not inevitably (Patterson et al., 2013). It is likely that the VGKC-complex antibodies bind to other antigenic targets that are yet to be identified in children (Hacohen et al., 2013). On the other hand, three patients were positive for CASPR2 antibodies, which have not been identified before in children, but were negative for VGKC-complex antibodies in the radioimmunoassay, so would have been missed with standard VGKC-complex-Ab screening.

None of the VGKC-complex or CASPR2 antibody-positive patients had a phenotype typical of limbic encephalitis, although most of them presented with focal seizures, suggesting that neuronal antibodies can be present beyond the spectrum of this recognized syndrome, especially in children. Two patients with positive NMDAR antibodies had epilepsy in the absence of classic autoimmune NMDAR-Ab encephalitis: one presented with focal dyscognitive seizures and one had mixed seizure types. This is similar to adult cases described with new-onset epilepsy associated with NMDAR Abs (Niehusmann et al., 2009; Brenner et al., 2013). In addition, two patients in our cohort, both presenting with generalized seizures, were positive for both NMDAR and VGKC-complex-Abs. The finding of two or more neuronal Abs is beginning to be recognized (Irani et al., 2010a; Pellkofer et al., 2010; Haberlandt et al., 2011) and may reflect a wider activation of the immune system, perhaps by infections or possibly as a secondary response to neuronal damage. NMDAR-Abs have recently been reported in one patient with MELAS (Finke et al., 2012), and secondary activation may be a possible explanation for the finding of two antibody-positive patients with known structural or metabolic cause for their epilepsy (cases 7 and 9).

Nine of the 11 positive patients with recorded follow-up had on going epilepsy and/or seizures, but unfortunately none of the 11 positive patients received immunotherapy. These patients did not develop long-term cognitive or behavioral impairment, although the follow-up was short and detailed neuropsychology was not done. Because this was a prospective observational cohort study, we can only hypothesize that immunotherapy, if given, might have improved the epilepsy outcome in these patients.

The control group consisted of children with various immunologic, oncologic, and medical conditions, and three

controls were positive for one of the tested antibodies, although they did not have apparent neurologic symptoms. This level of positivity appears to be higher than in adult control cohorts (e.g., McKnight et al., 2005; Brenner et al., 2013). However, few other reports have used childhood controls; indeed all children in this cohort had been hospitalized, and approximately one third of the patients had infectious/inflammatory conditions. The ability to develop antibodies in response to circulating pathogens or environmental factors may be higher in children, particularly in those who are already unwell. However, if antibody levels are sustained, it is possible that these children could be at risk of future neurologic dysfunction.

This study is the first to describe a relatively large prospective cohort of pediatric patients with new-onset seizures, apply the new ILAE system for the organization of seizures and epilepsies (as for Berg et al., 2010), and test them for neuronal antibodies within 6 months of seizure onset. We found that specific neuronal antibodies were more common, and these may play an etiologic role in children with epilepsy of unknown cause, indicating that the new ILAE system may “facilitate the identification of non-genetic determinants of epilepsy” (Berg 2010). We suggest that further antibody testing could be performed in pediatric epilepsy patients classified in this “unknown” category, particularly those with focal epilepsy; it has already been suggested that increasing the number of investigations should help to identify the etiologies of this group (van Campen et al., 2013). Moreover, because the new ILAE organization does not incorporate autoimmune forms in the epilepsy or etiology classification, the possibility of including “immune-mediated epilepsy” as a separate category (Brenner 2013) needs to be considered. However, it is also possible that the presence of neuronal antibodies are, in some circumstances, an epiphenomenon or secondary to structural damage or generalized immune activation. Further studies should concentrate on identifying antibodies in children with epilepsy of unknown cause closer to disease onset, with systematic testing of the effects of immune therapies, and applying the recent guidelines to determine whether these seizure-related antibodies define forms of “autoimmune epilepsy.”

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drafting/revising the manuscript. Dr Sukhvir Wright (SW): Technical work, acquisition of data, analysis and interpretation of data, statistical analysis, and drafting/revising the manuscript. Dr Deepak Gill (DG): Contribution to ILAE classification and revising the manuscript. Dr Fabienne Brilot (FB): Study supervision and funding (Sydney laboratory) and sample storage and management. Dr Patrick Waters (PW): Contribution of vital reagents and supervision and drafting/revising the manuscript. Dr K Peacock (KP): Contribution to patients' recruitment and classification and revising the manuscript. Associate Professor Peter Procopis (PP): Contribution to patients' recruitment and classification and revising the manuscript. Ms Anjan Nibber (AN): Technical work, acquisition of data, contribution of vital reagents, and drafting/revising the manuscript. Professor Angela Vincent (AV): Analysis and interpretation of data, study supervision and funding (Oxford laboratory) and drafting/revising the manuscript. Associate Professor R. Dale (RD): Study concept, acquisition of data, study supervision and funding (Sydney laboratory), analysis and interpretation of data, and drafting/revising the manuscript. Dr Bethan Lang (BL): Study concept, acquisition of data, study supervision and funding (Oxford laboratory), analysis and interpretation of data, and drafting/revising the manuscript.

## DISCLOSURES

AV, PW, BL, and the Nuffield Department of Clinical Neurosciences in Oxford receive royalties and payments for antibody assays. JS, SW, DG, FB, KP, PP, AN, and RD do not report any conflict of interest with respect to this study. We confirm that the authors have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## REFERENCES

- Barajas RF, Collins E, Cha S, Geschwind MD. (2010) Adult-onset drug-refractory seizure disorder associated with anti-voltage-gated potassium-channel antibody. *Epilepsia* 51:473–477.
- Beghi E, Carpio A, Forsgren L, Hesdorffer DC, Malmgren K, Sander JW, Tomson T, Hauser WA. (2010) Recommendation for a definition of acute symptomatic seizure. *Epilepsia* 51:671–675.
- Beleza P. (2012) Acute symptomatic seizures: a clinically oriented review. *Neurologist* 18:109–119.
- Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshe SL, Nordli D, Plouin P, Scheffer IE. (2010) Revised terminology and concepts for organisation of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology. *Epilepsia* 51:676–685.
- Bien CG, Scheffer IE. (2011) Autoantibodies and epilepsy. *Epilepsia* 52:18–22.
- Blume WT, Lüders HO, Mizrahi E, Tassinari C, van Emde Boas W, Engel J. (2001) Glossary of descriptive terminology for ictal semiology: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 42:1212–1218.
- Brenner T, Sills GJ, Hart Y, Howell S, Waters P, Brodie MJ, Vincent A, Lang B. (2013) Prevalence of neurologic autoantibodies in cohorts of patients with new and established epilepsy. *Epilepsia* 54:1028–35.
- Brigo F, Bongiovanni LG, Cerini R, Manganotti P, Storti M, Fiaschi A. (2011) Infliximab-related seizures: a first case study. *Epileptic Disord* 13:214–217.
- van Campen JS, Jansen FE, Brouwer OF, Nicolai J, Braun KP. (2013) Interobserver agreement of the old and the newly proposed ILAE epilepsy classification in children. *Epilepsia* 54:726–732.
- Dale RC, Brilot F, Fagan E, Earl J. (2009a) Cerebrospinal fluid neopterin in paediatric neurology: a marker of active central nervous system inflammation. *Dev Med Child Neurol* 51:317–323.
- Dale RC, Irani SR, Brilot F, Pillai S, Webster R, Gill D, Lang B, Vincent A. (2009b) NMDA receptor antibodies in pediatric dyskinetic encephalitis lethargica. *Ann Neurol* 66:704–709.
- Dhamija R, Renaud DL, Pittock SJ, McKeon A, Lachance DH, Nickels KC, Wirrell EC, Kuntz NL, King MD, Lennon VA. (2011) Neuronal voltage-gated potassium channel complex autoimmunity in children. *Pediatr Neurol* 44:275–281.
- Engel J, International League Against Epilepsy (ILAE) (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. (2006) Report of the ILAE classification core group. *Epilepsia* 47:1558–1568.
- Finke C, Prüss H, Scheel M, Ostendorf F, Harms L, Borowski K, Wandinger KP, Ploner CJ. (2012) Anti-NMDA receptor antibodies in a case of MELAS syndrome. *J Neurol* 259:582–584.
- Florence NR, Davis RL, Lam C, Szerka C, Zhou L, Ahmad S, Campen CJ, Moss H, Peter N, Gleichman AJ, Glaser CA, Lynch DR, Rosenfeld MR, Dalmau J. (2009) Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 66:11–18.
- Haberlandt E, Bast T, Ebner A, Holthausen H, Kluger G, Kravljanc R, Kröll-Seger J, Kurlemann G, Makowski C, Rostasy K, Tuschen-Hofstätter E, Weber G, Vincent A, Bien CG. (2011) Limbic encephalitis in children and adolescents. *Arch Dis Child* 96:186–191.
- Hacohen Y, Wright S, Waters P, Agrawal S, Carr L, Cross H, De Sousa C, Devile C, Fallon P, Gupta R, Hedderly T, Hughes E, Kerr T, Lascelles K, Lin JP, Philip S, Pohl K, Prabakar P, Smith M, Williams R, Clarke A, Hemingway C, Wassmer E, Vincent A, Lim MJ. (2013) Paediatric autoimmune encephalopathies: clinical features, laboratory investigations and outcomes in patients with or without antibodies to known central nervous system autoantigens. *JNNP* 84:748–55.
- Ilingworth MA, Hanrahan D, Anderson CE, O'Kane K, Anderson J, Casey M, de Sousa C, Cross JH, Wright S, Dale RC, Vincent A, Kurian MA. (2011) Elevated VGKC-complex antibodies in a boy with fever-induced refractory epileptic encephalopathy in school-age children (FIREs). *Dev Med Child Neurol* 53:1053–1057.
- Irani SR, Buckley C, Vincent A, Cockerell OC, Rudge P, Johnson MR, Smith S. (2008) Immunotherapy-responsive seizure-like episodes with potassium channel antibodies. *Neurology* 71:1647–1648.
- Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, Peles E, Buckley C, Lang B, Vincent A. (2010a) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 133:2734–2748.
- Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, Peles E, Buckley C, Lang B, Vincent A. (2010b) N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* 133:1655–1667.
- Irani S, Bien C, Lang B. (2011a) Autoimmune epilepsies. *Curr Opin Neurol* 24:146–153.
- Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, Schott JM, Armstrong RJ, Zagami A, Bleasel A, Somerville ER, Smith SM, Vincent A. (2011b) Fachiobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol* 69:892–900.
- Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshé SL, Perucca E, Wiebe S, French J. (2010) Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51:1069–1077.
- Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, Matà S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J. (2009) AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol* 65:424–434.
- Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, Cowell JK, Dalmau J. (2010) Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 9:776–785.
- Majoie M, de Baets M, Renier W, Lang B, Vincent A. (2006) Antibodies to voltage-gated potassium channels in epilepsy. *Epilepsy Res* 71:135–141.
- McKnight K, Jiang Y, Hart Y, Cavey A, Wroe S, Blank M, Shoenfeld Y, Vincent A, Palace J, Lang B. (2005) Serum antibodies in epilepsy and seizure-associated disorders. *Neurology* 65:1730–1736.
- Nabbout R. (2012) Autoimmune and inflammatory epilepsies. *Epilepsia* 53:58–62.
- Niehusmann P, Dalmau J, Rudlowski C, Vincent A, Elger CE, Rossi JE, Bien CG. (2009) Diagnostic value of N-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. *Arch Neurol* 66:458–464.

- Palace J, Lang B. (2000) Epilepsy: an autoimmune disease? *JNNP* 69:711–714.
- Panayiotopoulos CP. (2011) The new ILAE report on terminology and concepts for organization of epileptic seizures: a clinician's critical view and contribution. *Epilepsia* 52:2155–2160.
- Panayiotopoulos CP. (2012) The new ILAE report on terminology and concepts for the organization of epilepsies: critical review and contribution. *Epilepsia* 53:399–404.
- Patterson RW, Zandi MS, Armstrong R, Schott JM, Vincent A. (2013) The clinical relevance of positive voltage gated potassium channel (VGKC)-complex antibodies: experience from a tertiary referral centre. *JNNP*. In press.
- Pellkofer HL, Kuempfel T, Jacobson L, Vincent A, Derfuss T. (2010) Non-paraneoplastic limbic encephalitis associated with NMDAR and VGKC antibodies. *JNNP* 81:1407–1408.
- Peltola J, Kulmala P, Isojarvi J, Saiz A, Latvala K, Palmio J, Savola K, Knip M, Kernen T, Graus F. (2000) Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. *Neurology* 55:46–50.
- Quek AML, Britton JW, McKeon A, So E, Lennon VA, Shin C, Klein CJ, Watson RE, Kotsenas AL, Lagerlund TD, Cascino GD, Worrell GA, Wirrell EC, Nickels KC, Aksamit AJ, Noe KH, Pittock SJ. (2012) Autoimmune epilepsy: clinical characteristics and response to immunotherapy. *Arch Neurol* 69:582–593.
- Shinnar S. (2010) The new ILAE classification. *Epilepsia* 51:715–717.
- Suleiman J, Brenner T, Gill D, Brilot F, Antony J, Vincent A, Lang B, Dale RC. (2011a) VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology* 76:1252–1255.
- Suleiman J, Brenner T, Gill D, Troedson C, Sinclair AJ, Brilot F, Vincent A, Lang B, Dale RC. (2011b) Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies. *Dev Med Child Neurol* 53:1058–1060.
- Suleiman J, Brilot F, Lang B, Vincent A, Dale RC. (2013) Autoimmune epilepsy in children: case series and proposed guidelines for identification. *Epilepsia* 54:1036–1045.
- Vincent A, Bien CG, Irani SR, Waters P. (2011) Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* 10:759–772.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Seizures types at onset for the total cohort (as Blume 2001).

## FULL-LENGTH ORIGINAL RESEARCH

# Autoimmune epilepsy in children: Case series and proposed guidelines for identification

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### SUMMARY

**Purpose:** Antibodies against neuronal surface proteins are increasingly recognized in autoimmune central nervous system (CNS) disorders in which seizures are the main or an important feature. The disorders include antibody-associated limbic encephalitis and N-methyl-D-aspartate receptor (NMDAR) encephalitis; however, seizures of autoimmune etiology may exist beyond the spectrum of these recognized syndromes. Because these seizures are potentially treatable with immune therapy, guidelines are needed to help in their early recognition.

**Methods:** We describe 13 representative children seen at our tertiary institution over a period of 3.5 years with suspected autoimmune epilepsy. Autoimmune epilepsy was suspected clinically when there was any of the following: (1) recognizable syndromes such as NMDAR encephalitis or limbic encephalitis, (2) evidence of CNS inflammation in cerebrospinal fluid or on magnetic resonance imaging (MRI), (3) the presence of other autoimmune diseases, or (4) positive response to immunotherapy. We tested these patients for neuronal surface antibodies (voltage gated potassium channel [VGKC]-complex, leucine rich glioma inactivated 1 [LGII], contactin-associated protein-like 2 [CASPR2], and NMDAR) and glutamic acid decarboxylase (GAD) antibodies. We modified the *J Neurol Neurosurg Psychiatry*, 83, 2012, 638 guidelines that were designed to classify adults with neuronal surface antibody syndromes (NSAS), to be more appropriate for children with suspected autoimmune epilepsy. Using the modified guidelines, the 13 patients were classified into definite,

probable, possible, unlikely, or unknown autoimmune epilepsy according to the presence of neuronal surface or GAD antibodies, and the response to immune therapy when given.

**Key Findings:** Of the 13 patients, 11 were females, and the mean age was 6 years (range 1–13 years). Three patients had classical NMDAR encephalitis, two had VGKC encephalitis, two had limbic encephalitis with negative antibodies, three had epilepsy with other autoimmune diseases (one with high titer GAD antibodies), two had fever-induced refractory epileptic encephalopathy in school-aged children (FIRES), and one epileptic encephalopathy associated with VGKC antibodies. Seven patients of the 13 children with suspected autoimmune epilepsy were positive for neuronal surface antibodies (NMDAR, n = 3; VGKC-complex, n = 3; and GAD, n = 1). Immunotherapy was given to nine cases, and a positive response was more common in patients with positive neuronal surface antibodies (5/5) compared to those with negative antibodies (2/4). Applying the proposed guidelines, the classification of autoimmune epilepsy was definite in five, probable in one, possible in three, unlikely in two, and unknown in two patients.

**Significance:** Neuronal surface antibodies and GAD antibodies are present in a proportion of children with suspected autoimmune epilepsy and may define a treatable subgroup of childhood epilepsy. The proposed guidelines can be useful in the recognition of children with seizures of autoimmune etiology.

**KEY WORDS:** Epilepsy, Autoimmune, Antibodies, FIRES, VGKC, NMDAR, GAD, Pediatrics.

The association between autoantibodies and central nervous system (CNS) disease is increasingly recognized. Serum and cerebrospinal fluid (CSF) antibodies that bind to neuronal cell surface proteins including channels and recep-

tors have the potential to be pathogenic and cause CNS disease. By contrast onconeural antibodies are typically targeted against intracellular antigens and not thought to be directly pathogenic (Vincent et al., 2011; Bien et al., 2012). Recently antibodies that bind extracellularly and are associated with CNS disorders have been called “neuronal surface antibodies” (NSAbs) and the disorders associated with these NSAbs are called “neuronal surface antibody syndromes” (NSAS) (Zuliani et al., 2012). There are well-defined CNS syndromes associated with NSAbs where seizures are an

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important feature. Examples include *N*-methyl-D-aspartate receptor (NMDAR) encephalitis in which 76–83% of patients will have focal, focal dyscognitive, or generalized seizures (Dalmau et al., 2007, 2008, 2011; Irani & Vincent, 2011), and voltage-gated potassium channel (VGKC)-complex antibody associated autoimmune limbic encephalitis (including leucine rich glioma inactivated 1 [LGI1] and contactin-associated protein-like 2 [CASPR2] antibodies) in which patients often have temporal lobe seizures (Irani et al., 2010; Lai et al., 2010). In addition, faciobrachial dystonic seizures are seen in adults in association with LGI1 antibodies and often precede the onset of the limbic encephalitis (Irani et al., 2011). Other NSAbs are less frequently found in adults with limbic encephalitis such as alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and  $\gamma$ -aminobutyric acid B (GABA<sub>B</sub>) receptor antibodies (Lai et al., 2009; Lancaster et al., 2010; Boronat et al., 2011). Antibodies to glutamic acid decarboxylase (GAD) have been associated with limbic encephalitis (Malter et al., 2010). Although GAD is an intracellular antigen and therefore GAD Abs themselves may not be pathogenic, it is possible that unrecognized NSAbs coexist with GAD Abs (Zuliani et al., 2012).

Zuliani et al. proposed guidelines for the recognition, testing, and treatment of suspected autoimmune CNS disorders. They used clinical criteria, supportive features, neuronal antibody testing, and the response to immune therapy to classify patients into categories of definite, probable, and possible NSAS (Zuliani et al., 2012). Lancaster and Dalmau have proposed an alternative laboratory-based algorithm for identification and assessment of antibodies to neuronal cell-surface antigens using cell based assays as well as rat brain immunohistochemistry and cultures of neurons for serum and CSF antibody binding (Lancaster & Dalmau, 2012).

In children, NMDAR encephalitis is well described (Florance et al., 2009), whereas limbic encephalitis has been described in association with a number of different autoantibodies including VGKC-complex Abs (Haberlandt et al., 2011; Suleiman et al., 2011a). In children there are other epileptic conditions where immune-mediated mechanisms are suspected such as febrile infection-related epilepsy syndrome (van Baalen et al., 2010) or fever-induced refractory epileptic encephalopathy in school-aged children (Nabbout et al., 2010, 2011), both called fever-induced refractory epileptic encephalopathy in school-aged children (FIRES). Previous terms used to describe similar syndromes include devastating epileptic encephalopathy in school-aged children (DESC) (Mikaeloff et al., 2006) and acute encephalitis with refractory repetitive partial seizures (AERRPS) (Sakuma, 2009). These conditions are characterized by new-onset refractory focal status epilepticus, preceded by fever or infection in previously normal children, followed by a chronic phase of refractory focal epilepsy and severe neurologic impairment (Sakuma et al., 2010). The

cause of these conditions is unknown and underlying immune mechanisms have been proposed (Sakuma et al., 2010; Specchio et al., 2010; Nabbout et al., 2011) but not proven.

Herein we present a representative case series of 13 children suspected to have an autoimmune basis for their epilepsies. We propose modified guidelines for the recognition of autoimmune epilepsy and apply these guidelines to the 13 children with suspected autoimmune epilepsy to test their utility.

## METHODS

### Cases identification

Through our clinical practice at The Children Hospital at Westmead (CHW) we identified cases with seizures that may have an autoimmune etiology. The neurology department at CHW is a busy tertiary children's hospital that sees 300–400 children with new onset seizures per year, as well as other acute and chronic neurologic diseases in children. The patients presented in this cohort were discussed in detail by JS and RCD as they were suspected to have an autoimmune cause of their epilepsy (as defined below), and were investigated for neuronal surface antibodies. This cohort does not represent all children with encephalitis (of all etiologies) seen during this time ( $n = 33$ ) and are currently being tested in a separate study at this hospital. It is also likely that other autoimmune epilepsies were missed by the investigating team and were not tested for antibodies. Instead this cohort should be considered a representative sample of children with suspected autoimmune epilepsy, which were used to test the utility of the modified guidelines.

We suspected autoimmune epilepsy in children with acute or subacute onset of seizures once other causes (infection, structural, metabolic, or genetic) were excluded, and when any of the features described in Table 1 were present.

Herein we describe 13 representative patients seen over a three and a half year period (late 2008 to mid-2012), who we suspected may have an autoimmune cause for their epilepsy, and who had serum available for testing for neuronal antibodies. No patients have been previously reported except for case 5 (Suleiman et al., 2011a) and case 10 (Suleiman et al., 2011b), and these two cases were included as they test the utility of the guidelines. This study was approved by the hospital ethics committee.

Ten patients had serum testing in the acute phase of their illness, whereas in three the serum was from the chronic symptomatic phase. All samples were taken before immune therapy, if given. Antibody assays were all performed in Oxford, United Kingdom using previously published methods (Irani et al., 2010), apart from case 3, for which the assay was performed in National NMDAR antibody referral laboratory (Brisbane, Qld, Australia).



**Table 1. Criteria and supportive features to suspect autoimmune epilepsy in children with seizures**

|   |
|---|
| <p>The following two clinical criteria are used to suspect autoimmune epilepsy associated with NSAbs and GAD antibodies (both are needed)</p> <ol style="list-style-type: none"> <li>1 Acute or subacute (&lt;12 weeks) onset of symptoms.</li> <li>2 Exclusion of other causes (CNS infection, trauma, toxic, tumor, metabolic, previous CNS disease).</li> </ol> <p>The following supportive features would strengthen the suspicion of autoimmune epilepsy (patients should have at least 1 of the following):</p> <ol style="list-style-type: none"> <li>1 The presence of a well-defined clinical syndrome such as NMDAR or limbic encephalitis</li> <li>2 CNS inflammation manifested by at least one of:             <ol style="list-style-type: none"> <li>a CSF pleocytosis (defined as &gt;5 white cells/mm<sup>3</sup>) or presence of oligoclonal bands, elevated IgG index, or elevated neopterin (defined as &gt;30 nM)</li> <li>b MRI abnormality compatible with an inflammatory or autoimmune encephalitis including increased signal in the mesiotemporal lobe (LE-like syndrome)</li> <li>c Inflammatory neuropathology on biopsy</li> </ol> </li> <li>3 History of other antibody mediated condition (e.g., myasthenia gravis), organ specific autoimmunity or other autoimmune disorders.<sup>a</sup></li> <li>4 Response to immunotherapy</li> </ol> <p><sup>a</sup>It is recognized that epilepsy is more common in many autoimmune disorders including multiple sclerosis, systemic lupus erythematosus, type 1 diabetes mellitus (T1DM), celiac disease, and autoimmune thyroid disease (Vincent &amp; Crino, 2011).</p> |
|---|

**Table 2. Classification categories of suspected autoimmune epilepsy in children identified using the criteria and supportive features in Table 1 (Zuliani et al., modified)**

|  |
|--|
| <p>Classification categories expressing the likelihood of autoimmune epilepsy based on the presence of NSAbs and GAD Abs and the response to immunotherapy (see Fig. 1):</p> <p><i>Definite autoimmune epilepsy is present if:</i><br/>         Known NSAbs are present in serum or CSF<br/>         AND there is response to immunotherapy</p> <p><i>Probable autoimmune epilepsy is present if</i><br/>         Known NSAbs are present and no immunotherapy responsiveness demonstrated (immunotherapy unsuccessful or not given)<br/>         OR GAD antibodies are present AND there is response to immunotherapy</p> <p><i>Possible autoimmune epilepsy is present if known NSAbs are negative and</i><br/>         GAD antibodies are present and no immunotherapy responsiveness demonstrated (unsuccessful or not given)<br/>         OR GAD antibodies are negative and there is a response to immunotherapy</p> <p><i>Unlikely autoimmune epilepsy is present if</i><br/>         Known NSAbs and GAD are negative and there is no response to immunotherapy</p> <p><i>Unknown autoimmune epilepsy<sup>a</sup> is present if</i><br/>         Known NSAbs and GAD are negative and immunotherapy is not given</p> <p><sup>a</sup>Patients in this category may move to a different category if they receive immunotherapy, such as “possible” if they respond or “unlikely” if they did not respond to immunotherapy.</p> |
|--|

### Proposed modified guidelines

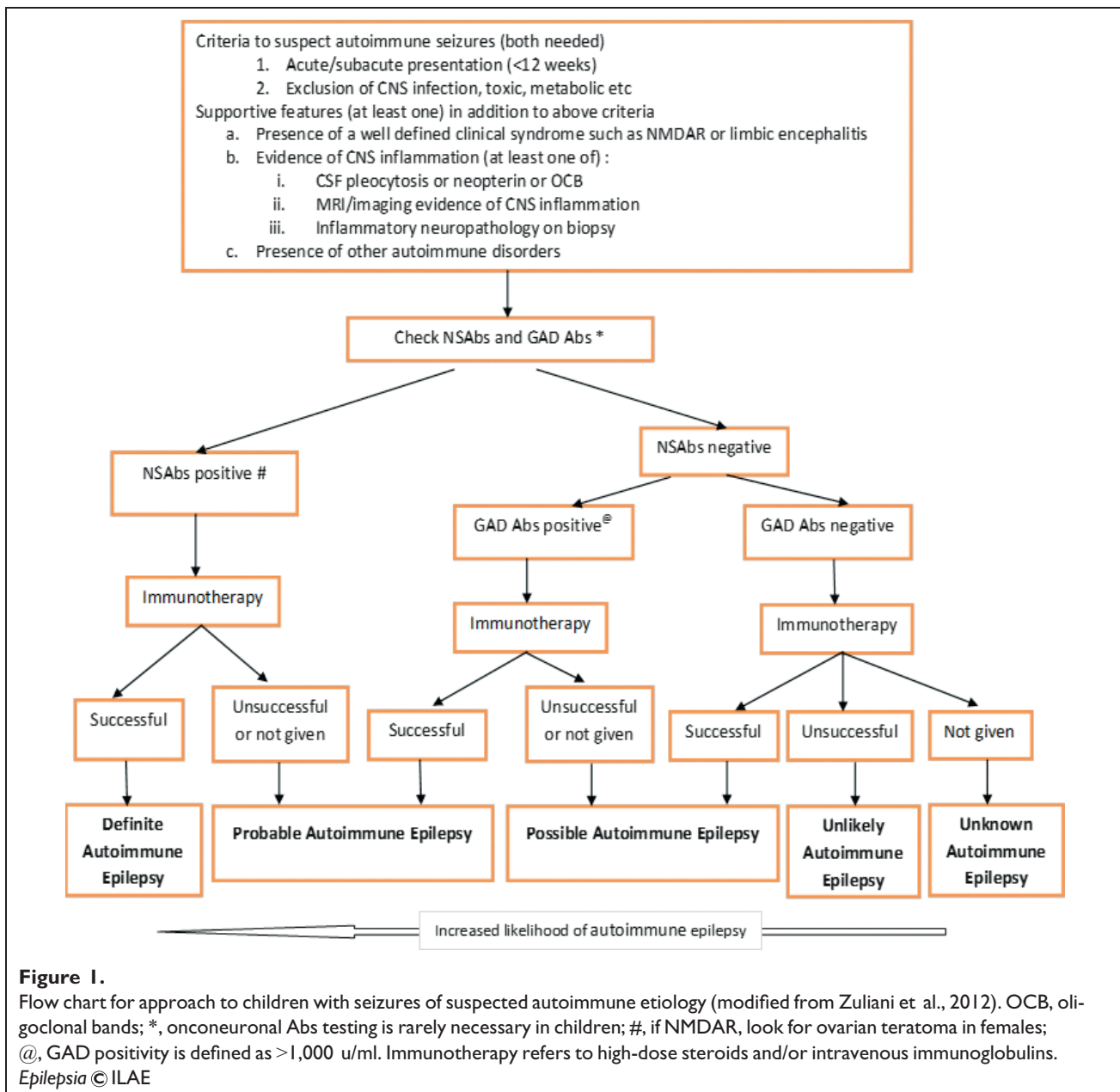
To improve recognition and diagnosis of children with suspected autoimmune epilepsy, we modified the guidelines proposed by Zuliani et al. for identification of children with neuronal surface antibodies syndromes (Table 1). Then, based on antibody testing and the response to immunotherapy (when given), we proposed five categories for classification (in descending order of likelihood of autoimmune epilepsy) including definite, probable, possible, unlikely, or unknown autoimmune epilepsy (Table 2, Fig. 1). We applied the modified guidelines to our 13 cases to test their usefulness (Table 3).

The main differences to the Zuliani et al. guidelines for adults include the following:

- 1 In children a paraneoplastic cause of epilepsy is very rare, and testing for onconeural antibodies is rarely necessary.

However; children with positive NMDAR Abs should be screened for ovarian teratomas.

- 2 In children fever and intercurrent infections are common and less likely to be a supportive feature for an autoimmune process as has been proposed in adults, so this was not used as one of the supportive features (Zuliani et al., 2012).
- 3 We used elevated CSF neopterin as an additional marker of CNS inflammation (Dale et al., 2009).
- 4 In Zuliani et al., abnormalities on functional imaging including hypermetabolism on positron emission tomography or hyperperfusion on single proton computed tomography were used as features to suggest CNS inflammation. However, there is inadequate research to demonstrate their ability to discriminate epilepsy etiologies in children and therefore we did not include these features.



**Figure 1.**

Flow chart for approach to children with seizures of suspected autoimmune etiology (modified from Zuliani et al., 2012). OCB, oligoclonal bands; \*, onconeural Abs testing is rarely necessary in children; #, if NMDAR, look for ovarian teratoma in females; @, GAD positivity is defined as >1,000 u/ml. Immunotherapy refers to high-dose steroids and/or intravenous immunoglobulins. *Epilepsia* © ILAE

- 5 Antibodies included in the guidelines are those available at international laboratories including antibodies against VGKC-complex proteins, LGI1 and CASPR2, NMDAR, and GAD. We did not include neuronal binding or neuropil antibody testing (using immunohistochemistry or immunofluorescence on rat brain tissue) for recognizable staining patterns (Lancaster & Dalmau, 2012), as these are done in research settings and are less available to clinicians routinely.
- 6 Response to immunotherapy was defined as significant clinical improvement of encephalopathy or reduction of seizures as judged by the treating clinicians. We accept the subjective nature of this, and the fact that there are a number of confounders that could be responsible for

improvements such as the concomitant change in anti-epileptic drugs.

- 7 We incorporated patients who did not receive immunotherapy into the classification either because an immune-mediated mechanism was not initially suspected or because of spontaneous improvement.

## RESULTS

The 13 patients with seizures of suspected autoimmune origin (11 female, age range 1–13 years, mean age 6 years) are presented in Table 3. All patients had other potential causes for their seizures excluded. All 13 patients had new-onset seizures and at least one supportive feature of CNS

**Table 3. Patients with suspected autoimmune epilepsy: clinical criteria, supportive features, and classification as per guidelines (see Fig. 1)**

| Case | Age (years)/sex | Epilepsy diagnosis  | Acute or sub-acute onset | Seizure type   | Associated features  | CSF inflammation (pleocytosis/OCB/neopterin) | MRI inflammatory changes | Presence of autoimmune/Ab mediated disease | NSAbs/GAD Abs       | Response to immune therapy       | Outcome                           | Guideline classification |
|------|-----------------|---------------------|--------------------------|--|--|--|--------------------------|--|---------------------|----------------------------------|-----------------------------------|--------------------------|
| 1    | 3/F             | NMDAR encephalitis  | +                        | Focal dyscognitive   | Encephalopathy, aphasia, dystonia, emotional lability, relapse | -/+  | -                        | -  | NMDAR CSF and serum | + (steroid, IVIG, mycophenolate) | Relapse, normal in between        | Definite                 |
| 2    | 6/M             | NMDAR encephalitis  | +                        | Focal dyscognitive   | Encephalopathy, agitation, chorea, dystonia                    | +/-/+  | -                        | -  | NMDAR CSF and serum | + (steroid and IVIG)             | Recovery                          | Definite                 |
| 3    | 7/F             | NMDAR encephalitis  | +                        | Focal dyscognitive   | Encephalopathy, agitation, irritability, dyskinesia, fever     | +/-/ND                                       | +                        | -  | NMDAR CSF and serum | + (steroids, IVIG)               | Recovery                          | Definite                 |
| 4    | 1/F             | VGKC encephalitis   | +                        | Focal dyscognitive, focal motor with automatism                | Encephalopathy, fever, respiratory infection                   | +/ND/+                                       | +                        | -  | VGKC serum (421 pm) | Not given                        | Recovery                          | Probable                 |
| 5    | 15/F            | VGKC encephalitis   | +                        | Focal, secondary generalized tonic-clonic, status epilepticus, | Encephalopathy, memory deficit, fever                          | +/-/+  | -                        | -  | VGKC serum (640 pm) | + (steroids, IVIG)               | Relapse, normal in between        | Definite                 |
| 6    | 12/F            | Limbic encephalitis | +                        | Focal dyscognitive   | Encephalopathy, lethargy, behavioral alteration                | ND   | +                        | +/- (ANA)                                  | Negative            | Not given                        | Cognitive, psychiatric impairment | Unknown                  |
| 7    | 15/F            | Limbic encephalitis | +                        | Focal dyscognitive, secondary generalized tonic-clonic         | Encephalopathy, cognitive deficits, fever                      | +/-/+  | -                        | -  | Negative            | + (steroid)                      | Recovery                          | Possible                 |

Continued

Table 3. Continued.

| Case | Age (years)/sex | Epilepsy diagnosis            | Acute or sub-acute onset | Seizure type                              | Associated features                        | CSF inflammation (pleocytosis/OCB/neopterin) | MRI inflammatory changes | Presence of autoimmune/Ab mediated disease | NSAbs/GAD Abs       | Response to immune therapy   | Outcome   | Guideline classification |
|------|-----------------|-------------------------------|--------------------------|---|--|--|--------------------------|--|---------------------|------------------------------|---|--------------------------|
| 8    | 3/M             | FIRES                         | +                        | Focal, status epilepticus                 | Encephalopathy, irritability, fever, rash, | -/-/+  | -                        | -  | Negative            | -(steroids, IVIG, rituximab) | Severe neurologic disability, refractory epilepsy | Unlikely                 |
| 9    | 8/F             | FIRES                         | +                        | Focal, secondary generalized              | Encephalopathy, headache, confusion, fever | +/-/+  | +/-                      | -  | Negative            | -(steroids)                  | Severe neurologic disability, refractory epilepsy | Unlikely                 |
| 10   | 1/F             | Epileptic encephalopathy      | +                        | Epileptic spasms                          | Encephalopathy, developmental delay        | -/+/+  | -                        | -  | VGKC serum (201 pm) | +(steroids)                  | Developmental delay                               | Definite                 |
| 11   | 13/F            | Suspected autoimmune epilepsy | +                        | Myoclonic, generalized tonic-clonic (JME) | Hyperthyroidism                            | ND   | -                        | +(Grave's disease and T1DM)                | Negative            | Not given                    | Ongoing epilepsy                                  | Unknown                  |
| 12   | 3/F             | Suspected autoimmune epilepsy | +                        | Focal dyscognitive                        | Myasthenia                                 | ND   | -                        | +(MG)                                      | Negative            | +(steroids)                  | Steroid dependent myasthenia                      | Possible                 |
| 13   | 4/F             | Suspected autoimmune epilepsy | +                        | Absence                                   | Ataxia                                     | -/ND/ND                                      | No                       | +(T1DM)                                    | GAD (3,000 U/ml)    | Not given                    | Cognitive impairment, ongoing epilepsy            | Possible                 |

FIRES, fever-induced refractory epileptic encephalopathy in school-aged children; JME, juvenile myoclonic epilepsy; OCB, oligoclonal bands; ND, not done; T1DM, type 1 diabetes mellitus; MG, myasthenia gravis; NSAb, neuronal surface antibody.  
 Encephalopathy is defined by the presence of acquired reduction in consciousness, cognitive dysfunction, or behavioral change lasting more than 24 h, and not related to the postictal state, +: present or positive, -: absent or negative.  
 Abnormal ranges: Pleocytosis: CSF white blood cells >5 cells/mm<sup>3</sup>, Neopterin elevated >30 pm, VGKC serum >100 pm, High titer cutoff for GAD antibodies in neurologic disease is 1,000 U/ml.

inflammation or the presence of other autoimmune diseases (Tables 1 and 2). Three patients had the clinical characteristics of NMDAR encephalitis (all with CSF and serum NMDAR antibodies), two had encephalitis associated with VGKC-complex antibodies, two had features suggestive of limbic encephalitis (with negative antibodies), three had epilepsy in association with other autoimmune diseases (one with GAD antibodies), two had FIRES, and one had epileptic encephalopathy with CNS inflammation (VGKC antibody positive).

Seven patients (53.9%) of the 13 were positive for one of the tested antibodies including NMDAR ( $n = 3$ ), VGKC-complex ( $n = 3$ ), and GAD ( $n = 1$ ). Immunotherapy was given in nine patients: five with positive neuronal antibodies and four negative. The immune therapy was steroids alone ( $n = 4$ ), steroids and intravenous immunoglobulin (IVIG) ( $n = 3$ ), steroids, IVIG and mycophenolate ( $n = 1$ ) and steroids, IVIG, and rituximab ( $n = 1$ ). All five patients with positive neuronal antibodies who received any immune therapy improved after receiving therapy, whereas only two of four with negative neuronal antibodies improved after receiving immune therapy. Three of the four patients who did not receive immunotherapy had poor outcome including ongoing epilepsy, and cognitive and psychiatric impairment (Table 3).

Patients were classified according to the proposed modified guidelines (Table 2, Fig. 1), and their classification is presented in Table 3. Five patients had definite, one had probable, three had possible, two had unlikely, and two had unknown autoimmune epilepsy.

We present the case histories for 8 of the 13 patients in details in the Data S1 as representative examples.

## DISCUSSION

The recognition of immune mechanisms in neurologic disorders is important as this can prompt early treatment and may lead to better outcomes. The identification of specific and potentially pathogenic NSAbs is increasing, and the spectrum of the clinical syndromes associated with NSAbs is widening (Zuliani et al., 2012). Recently guidelines have been developed to help in the diagnosis and management of adults with suspected NSAS (Zuliani et al., 2012). In children the lack of large studies regarding NSAbs and their related syndromes makes it harder to identify these cases; therefore, guidelines may help in the identification of NSAS particularly when seizures are an important feature.

In this paper, we describe 13 representative patients with seizures of suspected autoimmune etiology and we propose features for identification of these pediatric patients, and a classification system testing the strength of evidence of autoimmune epilepsy based on the presence of neuronal antibodies and response to immunotherapy.

There were some general features common to the cohort. Females were over-represented in this cohort, as is often

described in autoimmune disorders in general. The seizures were often focal, and generally occurred in association with encephalopathy or other features of CNS dysfunction.

Three cases had typical features of NMDAR encephalitis in children, as represented by case 3 description in the Data S1. The patients with NMDAR encephalitis generally had focal epilepsy, and the presence of psychiatric manifestations, behavior alteration, and movement disorder was a strong indicator of NMDAR encephalitis. However, it is possible that NMDAR Abs are present in children with epilepsy in the absence of the classic phenotype as has been described in adults (Niehusmann et al., 2009), and therefore testing for NMDAR Abs in children with suspected autoimmune seizures may provide further information about the spectrum of NMDAR antibody-associated disease.

Two cases had VGKC-complex Ab-associated encephalitis, characterized by fever-associated focal seizures and status epilepticus; one was previously reported (case 5) and the clinical phenotype of the second case (case 4) was similar to our previously reported pediatric patients with VGKC-complex Ab-associated encephalitis (Suleiman et al., 2011a). The seizure semiology was suggestive of temporal lobe onset, a finding that is commonly seen in both adults and children with this syndrome (Vincent et al., 2004; Suleiman et al., 2011a). In case 4, mycoplasma immunoglobulin M (IgM) was positive and was consistent with acute infection. Mycoplasma infection has been described in association with NMDAR encephalitis in children and may be a trigger of autoimmune CNS disorders (Florance et al., 2009). However, mycoplasma pneumonia is a common respiratory infection in children and positive mycoplasma serology may therefore be incidental in some patients (Waites & Talkington, 2004). Antibodies against LGI1 or CASPR2, which have been identified as the target of VGKC-complex Abs in adults, were negative in this case, a finding that is common in children with positive VGKC-complex Abs. It is possible that in children, VGKC-complex Abs are targeted against other antigens in the VGKC-complex that are yet to be identified. Patients with VGKC-complex Ab-associated encephalitis often respond to immune therapy, but spontaneous improvement can also occur (Irani et al., 2010) as was the case in this patient.

Case 6 had a syndrome of limbic encephalitis; however, NSAbs and GAD Abs were negative, possibly due to late testing and because the patient received no immunotherapy, the classification was "unknown." Early recognition, testing, and treatment might have improved her outcome. Case 7 had a limbic encephalitis syndrome and was negative for NSAbs but responded to immune therapy (classification possible). Antibodies against AMPAR and GABA<sub>B</sub>R (not tested) or other unrecognized NSAbs could be the cause of limbic encephalitis in these patients. The diagnosis of limbic encephalitis can be challenging in children, where its existence is reported but probably underrecognized (Haberlandt et al., 2011). The diagnosis of limbic encephalitis is

partly clinical, with new-onset temporal lobe seizures and cognitive disturbance sometimes associated with radiologic mesial temporal or hippocampal changes. Because hippocampal signal change is described in a proportion of children with febrile status epilepticus (Shinnar et al., 2012), it is difficult to discriminate radiologic seizure-induced hippocampal swelling from limbic encephalitis.

Cases 8 and 9 were typical of “FIRES” (van Baalen et al., 2010; Nabbout et al., 2011). Neuronal antibodies were negative, and there was no response to immunotherapy in both patients. The absence of antibodies and the negative response to immune therapy make an autoimmune etiology “unlikely.” Negative response to immunotherapy has been reported in a series of seven cases of FIRES, and NSAbs were negative in the tested patients (three were tested for VGKC-complex Abs and one for NMDAR Abs) (Howell et al., 2012). In addition a series of 12 children with FIRES was negative for neuronal surface antibodies and GAD (van Baalen et al., 2012). There is one report of a boy with positive VGKC antibodies associated with FIRES who benefited from immunotherapy (Illingworth et al., 2011); however, this case did not have a typical course of FIRES and it is possible that the case had VGKC-complex antibody-associated encephalitis instead. The markers of CNS inflammation seen in our two cases (8 and 9) have been reported in the acute phase of FIRES, and may be explained by the extreme high seizure load, seizure-related neuronal injury, or cytokine release (Howell et al., 2012). Rather than an autoimmune epilepsy syndrome, FIRES may be a genetic channelopathy or a chronic epilepsy syndrome with explosive onset (Ismail & Kossoff, 2011; Howell et al., 2012).

Three of our cases had epilepsy in association with other autoimmune diseases including type 1 diabetes mellitus (T1DM) and autoimmune thyroid disease (case 11), anti MuSK myasthenia gravis (case 12), and T1DM and possible autoimmune ataxia (case 13).

T1DM is a T cell-mediated autoimmune disorder, and there is an increased prevalence of epilepsy in children with this disease (Schober et al., 2012).

Seizures can occur in Hashimoto’s encephalopathy, which is a rare association of autoimmune Hashimoto’s thyroiditis associated with Abs against thyroid peroxidase and thyroglobulin (Castillo et al., 2006). Patients described with Hashimoto encephalopathy present with broad clinical manifestations and are classically reported to be steroid responsive. The role of thyroid antibodies in Hashimoto encephalopathy is uncertain, and the term “steroid responsive encephalopathy associated with autoimmune thyroiditis” (SREAT) has been used to reflect the hypothesis that Hashimoto encephalopathy may be caused by unidentified neuronal autoantibodies (Castillo et al., 2002; Schauble et al., 2003).

Graves’ disease is an antibody mediated autoimmune disorder and juvenile myoclonic epilepsy (JME) has been previously associated with Grave’s disease, and may be due to thyroxine causing a lower seizure threshold (Su et al.,

1993). Our case 11 was diagnosed to have an idiopathic myoclonic epilepsy (JME) based on her age, seizure phenotype, and EEG abnormality. JME is considered to be a genetic epilepsy, and indeed in this case there was limited evidence that the epilepsy was autoimmune despite the presence of other autoimmune diseases, and her classification was “unknown” as she was negative for NSAbs and received no immunotherapy.

Seizures in association with anti MuSK Ab myasthenia gravis are rare but have been reported in an adult patient (Bhagavati et al., 2007). Case 12 had anti-MuSK Ab associated myasthenia gravis and concurrent focal epilepsy. Her seizures did not respond to carbamazepine but improved when high dose steroids were used to treat her myasthenia gravis. It is possible that myasthenia gravis and epilepsy in our patient is a chance association, although both clinical entities presented, remitted and relapsed concurrently.

Case 13 had seizures in the context of T1DM. This patient had an acute transient ataxia followed by chronic epilepsy, with very high GAD antibodies. GAD antibodies are associated with a variety of CNS syndromes including stiff person syndrome, immune ataxia, epilepsy, and limbic encephalitis (Honnorat et al., 2001; Saiz et al., 2008; Malter et al., 2010). In our patient the immune-mediated ataxia, cognitive impairment, focal epilepsy, and high GAD antibodies were supportive of the autoimmune epilepsy hypothesis.

Patients with epilepsy and other systemic autoimmune diseases may have other as-yet-unidentified NSAbs. However, other explanations for increased epilepsy incidence in systemic autoimmune disorders include incidental coexistence, a common genetic predisposition, or secondary effects of the primary disease (Vincent & Crino, 2011).

One important feature of the adult guidelines is that response to immunotherapy is used as a retrospective feature to help with classification. In other words the “guideline classification” cannot be completed until immunotherapy is used. Our modified guidelines partly address this issue and incorporate patients who did not receive immunotherapy. In our case, series some patients did not receive immunotherapy either because an autoimmune etiology was not initially suspected at presentation or due to spontaneous improvement without the need for immunotherapy. A positive response to immunotherapy was more common in patients who had positive NSAb (five of five given immunotherapy) compared to those who were NSAb negative (two of four). However, in a recent study of 48 children with suspected autoimmune encephalitis, only 21 had specific antibodies detected, and beneficial treatment responses were seen in both antibody-positive and antibody-negative groups (Hacohen et al., 2012).

In our clinical practice over the last few years we have been increasingly using immunotherapy empirically once an underlying immune-mediated disorder is suspected while awaiting the specific investigations. Children suspected of potential autoimmune epilepsy undergo investigations to



exclude infectious, toxic, metabolic, or genetic causes, and neuronal surface and GAD antibodies are requested. While awaiting the results of the neuronal antibodies, empiric immunotherapy may be commenced if the clinical syndrome is severe and impairing. We suggest that immunotherapy be used early in the disease course to optimize its potential effect. The regimen we have been using includes intravenous pulse methylprednisolone at 30 mg/kg/day for 3 days followed by a tapering course of oral prednisolone (variable duration of weeks to months according to the disorder), often in conjunction with intravenous immunoglobulins at 2 g/kg given over 2 days. Patients with partial response or no response after 1–3 weeks may receive further doses of intravenous immunoglobulins or plasma exchange if the condition is severe and concerning, and the autoimmune hypothesis remains possible. Patients who fail to respond or who have a partial response may be considered for second-line therapy, such as rituximab or cyclophosphamide. However the side effect profile of these drugs is more concerning, so a “risk versus benefit” assessment is necessary. In our case series immunotherapy was generally tolerated well, particularly when given short term (such as the NMDAR encephalitis cases). Two patients developed significant side effects attributed to immunotherapy including behavioral alteration with prolonged steroid use (case 12) and prolonged hypogammaglobulinemia requiring IVIG replacement presumed to be secondary to rituximab (case 8), a finding that has been described previously (Makatsori et al., 2012). Some patients with seizures of autoimmune etiology can have complete recovery without immunotherapy (similar to case 4); however, it is hard to predict which cases will spontaneously recover, and therefore early immunotherapy is suggested when the patient is severely impaired. Similar treatment regimens have been used in adults with VGKC Ab-positive encephalitis with good effect (Reid et al., 2009; Wong et al., 2010). Although plasma exchange is used commonly in adults, the use of plasma exchange in children as a modality of immune therapy is limited due to its invasiveness, the need for intensive care treatment, and potential side effects.

Although a positive response to immunotherapy supports immune-mediated mechanisms, steroids (and IVIG to a lesser extent) are used in the treatment of refractory and severe epilepsies that are not proven to be autoimmune.

In conclusion, autoimmune mechanisms play an important role in a proportion of children presenting with seizures. We propose guidelines that may help clinicians in the approach to identify children with suspected autoimmune seizures. Although helpful, the guidelines are not perfect and represent only an attempt to identify and classify these patients. These guidelines do not predict treatment responsiveness or outcome. Future studies may improve the understanding of clinical phenotypes of autoimmune epilepsy in children and help further develop syndrome-specific and treatment-oriented guidelines.

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## DISCLOSURE

AV and the Department of Clinical Neurology in Oxford receive royalties and payments for antibody assays, and AV is the named inventor on patent application WO/2010/046716 entitled “Neurological Autoimmune Disorders.” The patent has been licensed to Euroimmun AG for the development of assays for LGI1 and other VGKC-complex antibodies. AV and BL are coinventors and may also receive future royalties. None of the other authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## REFERENCES

- Bhagavati S, Maccabee PJ, Chari G. (2007) Is cerebral involvement an occasional feature of muscle-specific kinase antibody-positive syndrome? *Eur J Neurol* 14:E21–E22.
- Bien CG, Vincent A, Barnett MH, Becker AJ, Blumcke I, Graus F, Jellinger KA, Reuss DE, Ribalta T, Schlegel J, Sutton I, Lassmann H, Bauer J. (2012) Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* 135:1622–1638.
- Boronat A, Sabater L, Saiz A, Dalmau J, Graus F. (2011) GABA(B) receptor antibodies in limbic encephalitis and anti-GAD-associated neurologic disorders. *Neurology* 76:795–800.
- Castillo PR, Boeve BF, Caselli RJ, Vernino SA, Lucchinetti C, Swanson JW, Noseworthy JH, Aksamit AJ, Flemming KD, Hunder GG, Fatourechi V, Mokri B, Lennon VA. (2002) Steroid-responsive encephalopathy associated with thyroid autoimmunity: clinical and laboratory findings. *Neurology* 58:A248.
- Castillo P, Woodruff B, Caselli R, Vernino S, Lucchinetti C, Swanson J, Noseworthy J, Aksamit A, Carter J, Sirven J, Hunder G, Fatourechi V, Mokri B, Drubach D, Pittock S, Lennon V, Boeve B. (2006) Steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Arch Neurol* 63:197–202.
- Dale RC, Brilot F, Fagan E, Earl J. (2009) Cerebrospinal fluid neopterin in paediatric neurology: a marker of active central nervous system inflammation. *Dev Med Child Neurol* 51:317–323.
- Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR. (2007) Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* 61:25–36.
- Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng XY, Lai MZ, Dessain SK, Rosenfeld MR, Balice-Gordon R, Lynch DR. (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 7:1091–1098.
- Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. (2011) Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* 10:63–74.
- Florance NR, Davis RL, Lam C, Szperka C, Zhou L, Ahmad S, Campen CJ, Moss H, Peter N, Gleichman AJ, Glaser CA, Lynch DR, Rosenfeld MR, Dalmau J. (2009) Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 66:11–18.
- Haberlandt E, Bast T, Ebner A, Holthausen H, Kluger G, Kravljanc R, Kroll-Seger J, Kurlemann G, Makowski C, Rostasy K, Tuschen-Hofstatter E, Weber G, Vincent A, Bien CG. (2011) Limbic encephalitis in children and adolescents. *Arch Dis Child* 96:186–191.
- Hacohen YWS, Waters P, Agrawal S, Carr L, Cross H, De Sousa C, DeVile C, Fallon P, Gupta R, Hedderly T, Hughes E, Kerr T, Lascelles K, Lin J-P, Philip S, Pohl K, Prabakar P, Smith M, Williams R, Clarke A, Hemingway C, Wassmer E, Vincent A, Lim M. (2012) Paediatric autoimmune encephalopathies: clinical features, laboratory

- investigations and outcomes in patients with or without antibodies to known central nervous system (CNS) autoantigens. *J Neurol Neurosurg Psychiatr*. In press.
- Honnorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, Maestre J, Fabien N, Vighetto A, Casamitjana R, Thivolet C, Tavolato B, Antoine JC, Trouillas P, Graus F. (2001) Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies – study of 14 patients. *Arch Neurol* 58:225–230.
- Howell KB, Katanyuwong K, Mackay MT, Bailey CA, Scheffer IE, Freeman JL, Berkovic SF, Harvey AS. (2012) Long-term follow-up of febrile infection-related epilepsy syndrome. *Epilepsia* 53:101–110.
- Illingworth MA, Hanrahan D, Anderson CE, O’Kane K, Anderson J, Casey M, de Sousa C, Cross JH, Wright S, Dale RC, Vincent A, Kurian MA. (2011) Elevated VGKC-complex antibodies in a boy with fever-induced refractory epileptic encephalopathy in school-age children (FIRES). *Dev Med Child Neurol* 53:1053–1057.
- Irani SR, Vincent A. (2011) NMDA receptor antibody encephalitis. *Curr Neurol Neurosci Rep* 11:298–304.
- Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, Peles E, Buckley C, Lang B, Vincent A. (2010) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan’s syndrome and acquired neuromyotonia. *Brain* 133:2734–2748.
- Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, Schott JM, Armstrong RJE, S. Zagami A, Bleasel A, Somerville ER, Smith SMJ, Vincent A (2011) Faciobrachial dystonic seizures precede Lgl1 antibody limbic encephalitis. *Ann Neurol* 69:892–900.
- Ismail FY, Kossoff EH. (2011) AERRPS, DESC, NORSE, FIRES: multi-labeling or distinct epileptic entities? *Epilepsia* 52:E185–E189.
- Lai MZ, Hughes EG, Peng XY, Zhou L, Gleichman AJ, Shu H, Mata S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J. (2009) AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol* 65:424–434.
- Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, Cowell JK, Dalmau J. (2010) Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 9:776–785.
- Lancaster E, Dalmau J. (2012) Neuronal autoantigens-pathogenesis, associated disorders and antibody testing. *Nat Rev Neurol* 8: 380–390.
- Lancaster E, Lai MZ, Peng XY, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J. (2010) Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* 9:67–76.
- Makatsori M, Manson AL, Verma N, Leandro M, Gurugama P, Longhurst H, Grigoriadou S, Buckland M, Kiani-Alikhan S, Hanson S, Ibrahim MA, Chee R, Kanfer E, Grimbacher B, Seneviratne SL. (2012) Post-rituximab prolonged hypogammaglobulinemia requiring intravenous immunoglobulin replacement therapy (IVIG). *J Clin Immunol* 32:283.
- Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. (2010) Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann Neurol* 67:470–478.
- Mikaeloff Y, Jambaque I, Hertz-Pannier L, Zamfirescu A, Adamsbaum C, Plouin P, Dulac O, Chiron C. (2006) Devastating epileptic encephalopathy in school-aged children (DESC): a pseudo encephalitis. *Epilepsy Res* 69:67–79.
- Nabbout R, Mazzuca M, Hubert P, Peudennier S, Allaire C, Flurin V, Aberastury M, Silva W, Dulac O. (2010) Efficacy of ketogenic diet in severe refractory status epilepticus initiating fever induced refractory epileptic encephalopathy in school age children (FIRES). *Epilepsia* 51:2033–2037.
- Nabbout R, Vezzani A, Dulac O, Chiron C. (2011) Acute encephalopathy with inflammation-mediated status epilepticus. *Lancet Neurol* 10:99–108.
- Niehusmann P, Dalmau J, Rudlowski C, Vincent A, Elger CE, Rossi JE, Bien CG. (2009) Diagnostic value of N-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. *Arch Neurol* 66:458–464.
- Reid JM, Foley P, Willison HJ. (2009) Voltage-gated potassium channel-associated limbic encephalitis in the west of Scotland: case reports and literature review. *Scott Med J* 54:27–31.
- Saiz A, Blanco Y, Sabater L, Gonzalez F, Bataller L, Casamitjana R, Ramio-Torrenta L, Graus F. (2008) Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain* 131:2553–2563.
- Sakuma H. (2009) Acute encephalitis with refractory, repetitive partial seizures. *Brain Dev* 31:510–514.
- Sakuma H, Awaya Y, Shiomi M, Yamanouchi H, Takahashi Y, Saito Y, Sugai K, Sasaki M. (2010) Acute encephalitis with refractory, repetitive partial seizures (AERRPS): a peculiar form of childhood encephalitis. *Acta Neurol Scand* 121:251–256.
- Schauble B, Castillo PR, Boeve BF, Westmoreland BF. (2003) EEG findings in steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Clin Neurophysiol* 114:32–37.
- Schober E, Otto KP, Dost A, Jorch N, Holl R, German Austrian DPVI, Diab BCN. (2012) Association of epilepsy and type 1 diabetes mellitus in children and adolescents: is there an increased risk for diabetic ketoacidosis? *J Pediatr* 160:662–667.
- Shinnar S, Bello JA, Chan S, Hesdorffer DC, Lewis DV, Macfall J, Pellock JM, Nordli DR, Frank LM, Moshe SL, Gomes W, Shinnar RC, Sun S. (2012) MRI abnormalities following febrile status epilepticus in children: the FEBSTAT study. *Neurology* 79:871–877.
- Specchio N, Fusco L, Claps D, Vigeveno F. (2010) Epileptic encephalopathy in children possibly related to immune-mediated pathogenesis. *Brain Dev* 32:51–56.
- Su YH, Izumi T, Kitsu M, Fukuyama Y. (1993) Seizure threshold in juvenile myoclonic epilepsy with graves-disease. *Epilepsia* 34:488–492.
- Suleiman J, Brenner T, Gill D, Brilot F, Antony J, Vincent A, Lang B, Dale RC. (2011a) VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology* 76:1252–1255.
- Suleiman J, Brenner T, Gill D, Troedson C, Sinclair AJ, Brilot F, Vincent A, Lang B, Dale RC. (2011b) Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies. *Dev Med Child Neurol* 53: 1058–1060.
- van Baalen A, Hausler M, Boor R, Rohr A, Sperner J, Kurlemann G, Panzer A, Stephani U, Kluger G. (2010) Febrile infection-related epilepsy syndrome (FIRES): a nonencephalitic encephalopathy in childhood. *Epilepsia* 51:1323–1328.
- van Baalen A, Hausler M, Plecko-Startinig B, Strautmanis J, Vlaho S, Gebhardt B, Rohr A, Abicht A, Kluger G, Stephani U, Probst C, Vincent A, Bien CG. (2012) Febrile infection-related epilepsy syndrome without detectable autoantibodies and response to immunotherapy: a case series and discussion of epileptogenesis in FIRES. *Neuropediatrics* 43:209–216.
- Vincent A, Crino PB. (2011) Systemic and neurologic autoimmune disorders associated with seizures or epilepsy. *Epilepsia* 52:12–17.
- Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J, Vincent A, Buckley C, Schott JM, Baker I, Dewar B-K, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J. (2004) Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 127: 701–712.
- Vincent A, Bien CG, Irani SR, Waters P. (2011) Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* 10:759–772.
- Waite KB, Talkington DF (2004) Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev* 17:697–728.
- Wong SH, Saunders MD, Larner AJ, Das K, Hart IK. (2010) An effective immunotherapy regimen for VGKC antibody-positive limbic encephalitis. *J Neurol Neurosurg Psychiatry* 81:1167–1169.
- Zuliani L, Graus F, Giometto B, Bien C, Vincent A. (2012) Central nervous system neuronal surface antibody associated syndromes: review and guidelines for recognition. *J Neurol Neurosurg Psychiatry* 83:638–645.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Clinical case histories of eight of the patients are described in detail.



# Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies

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## ABBREVIATIONS

|        |                                       |
|--------|---------------------------------------|
| CASPR2 | Contactin-associated protein 2        |
| GAD    | Glutamic acid decarboxylase           |
| LGI1   | Leucine-rich glioma-inactivated 1     |
| NMDAR  | <i>N</i> -methyl-D-aspartate receptor |
| OCB    | Oligoclonal band                      |
| VGKC   | Voltage-gated potassium channel       |

Autoantibodies that bind to voltage-gated potassium-channel complex proteins (VGKC-complex antibodies) occur frequently in adults with limbic encephalitis presenting with cognitive impairment and seizures. Recently, VGKC-complex antibodies have been described in a few children with limbic encephalitis, and children with unexplained encephalitis presenting with status epilepticus. We report a case of infantile-onset epileptic spasms and developmental delay compatible with epileptic encephalopathy. Our patient was a female infant, aged 4 months at presentation. She had evidence of immune activation in the central nervous system with elevated cerebrospinal fluid neopterin and mirrored oligoclonal bands, which prompted testing for autoantibodies. VGKC-complex antibodies were elevated (201 pmol/L, normal <100), but extended antibody testing, including leucine-rich glioma-inactivated 1 (LGI1) and contactin-associated protein 2 (CASPR2), was negative. The patient showed a partial response to steroid treatment, which was started late in the disease course. On review at 13 months of age, her development was consistent with an age of 5 to 6 months. These results suggest that VGKC-complex antibodies might represent a marker of immune therapy responsiveness in a subgroup of patients with infantile epileptic encephalopathy.

Epileptic encephalopathies are a group of conditions in which cognitive and neurological functions deteriorate in association with epileptic activity.<sup>1</sup> Epileptic encephalopathy can occur at any age; however, it is most common and severe in infancy and early childhood, when global and profound cognitive impairment might occur.<sup>2</sup> The encephalopathy might result from the underlying cause, the epileptic process, or a combination of both. Epileptic spasms are a severe form of epilepsy and epileptic encephalopathy that usually affect infants and was previously called infantile spasms.<sup>2</sup> Epileptic spasms are difficult to treat and are typically associated with delayed development.<sup>3</sup> Interictal electroencephalography (EEG) in patients with epileptic spasms often shows a characteristic chaotic and high-voltage pattern, which, when typical, is called hypsarrhythmia.<sup>4</sup> Epileptic encephalopathy and epileptic spasms can be caused by multiple aetiologies including structural, metabolic, and genetic causes;<sup>1,4</sup> however, in some cases, no aetiology can be found.<sup>4</sup>

There is increasing interest in the role of inflammation in epilepsy and status epilepticus.<sup>5</sup> The immune process can be primary and cause seizures or, alternatively, inflammation can be secondary to seizures themselves.<sup>5</sup> Antineuronal antibodies

binding to *N*-methyl-D-aspartate receptor (NMDAR), glutamic acid decarboxylase (GAD), and voltage-gated potassium channels (VGKCs) have been associated with a spectrum of syndromes in the central nervous system including seizures and limbic encephalitis, and are proposed to be causative.<sup>6–10</sup> Limbic encephalitis associated with VGKC-complex antibodies classically presents with seizures and cognitive impairment and is immune responsive.<sup>6</sup> These antibodies were originally thought to be directed towards the VGKCs themselves, but it is now clear that most bind to proteins that are part of the VGKC complex, particularly leucine-rich glioma-inactivated 1 (LGI1) and contactin-associated protein 2 (CASPR2).<sup>11,12</sup> Antibodies to LGI1 are particularly found in patients with immune-responsive limbic encephalitis and in the recently described epileptic syndrome, facio-brachial dystonic seizures.<sup>13</sup> Recently, VGKC-complex antibodies have been reported in children with limbic encephalitis,<sup>14</sup> and in encephalitis presenting with status epilepticus.<sup>15</sup> These paediatric patients had a variable outcome including temporal lobe epilepsy and cognitive impairment<sup>15</sup> but they were not treated promptly with immune therapy. VGKC-complex autoimmunity has also been recently described in children with

various neurological manifestations including one with early-onset symptomatic generalized epilepsy associated with mesotemporal magnetic resonance imaging (MRI) changes.<sup>16</sup> These findings support the importance of autoimmune mechanisms in some children with epilepsy.

We report a female with early-onset epileptic encephalopathy and epileptic spasms who had elevated cerebrospinal fluid (CSF) neopterin and mirrored oligoclonal bands (OCBs) suggestive of immune activation.<sup>17</sup> The patient had elevated VGKC-complex antibodies and showed a partial response to steroid therapy.

## METHOD

Over the past 5 years we have measured CSF neopterin<sup>17</sup> and CSF OCBs in all patients who undergo CSF testing. Of 19 patients with epileptic encephalopathy tested, only two had raised neopterin and positive OCBs. One case, which is not reported here, had immunodeficiency and epileptic encephalopathy. The second had unexplained epileptic encephalopathy with epileptic spasms and is described here. As we suspected a possible autoimmune process in this reported case, we retrospectively tested serum for autoantibodies against neuronal antigens including VGKC, NMDAR, GAD, LGI1, and CASPR2 using serum collected 10 months after seizure onset. The study of autoantibodies in children with epilepsy was approved by the Children's Hospital at Westmead ethics committee. Informed consent for publication of this report was obtained from the parents of the patient.

## CASE REPORT

A female of South Pacific origin was referred to our hospital with epileptic spasms. She was delivered at term with no complications. She started to have epileptic spasms at the age of 4 months that occurred in clusters of up to four per day (one to four spasms per cluster). Vigabatrin was started by the referring doctors, which helped initially; however, the spasms recurred and did not respond to increasing doses of vigabatrin (maximum dose of 100mg/kg/day). The patient had significant developmental delay and on review at 13 months of age her development was consistent with an age of 5 to 6 months. Examination revealed truncal hypotonia, but normal neurological examination otherwise. She developed eczema at the age of 3 to 4 months, which was resistant to topical treatment.

EEG showed slow background with high amplitude multi-focal spike and slow-wave complexes compatible with 'modified hypsarrhythmia' and consistent with epileptic encephalopathy. Brain MRI showed mild diffuse cerebral atrophy and mild delay in white matter myelination. The following investigations were normal: blood for lactate, ammonia, blood count, thyroid, renal and liver function tests, biotinidase, transferrin isoforms, very-long-chain fatty acids, white cell enzymes and plasma amino acids, urine for metabolic screen, CSF for glucose, lactate, amino acids, cells, and culture. CSF neopterin was mildly elevated at 33.3nmol/L (normal<28) and mirrored OCBs were detected in CSF and serum.

Pyridoxine and biotin were started at age 13 months with no effect. Levetiracetam was added with some benefit and

## What this paper adds

- We present a female infant with epileptic spasms and epileptic encephalopathy who had immune activation in the central nervous system.
- This is the first report of specific antibodies in a patient with epileptic spasms and epileptic encephalopathy.
- Immune mechanisms might be important in some of these cases and early immune therapy might improve outcome.

vigabatrin was stopped. Progress EEG on levetiracetam showed improvement in the background activity and persistence of multifocal epileptiform discharges.

Over the next few months, the patient had no obvious clinical seizures; however, she made no progress in her development and her eczema deteriorated. She lived internationally and was 2 years old at the time of reassessment. The patient had severe eczema and abnormal dyskinetic movements of both arms. Her central hypotonia persisted. She was able to roll from supine to prone, but was not reaching for objects. She was fixing and following, vocalizing, but had no words. Her EEG remained encephalopathic with a modified hypsarrhythmic pattern, and repeat MRI was unchanged. Oral prednisolone at 40mg daily was started according to the United Kingdom Infantile Spasms Study (UKISS).<sup>3,18</sup> This was followed by improvement in encephalopathic state within a few days with improved awareness and interactions. Her eczema also improved. Repeat EEG 8 days after starting steroids showed marked improvement.

Retrospective testing of serum taken at 13 months of age revealed antibodies to VGKC-complex (201pmol/L, normal<100pmol/L), but negative NMDAR, GAD, LGI1, and CASPR2 antibodies.

## DISCUSSION

The recognition of autoimmune mechanisms in children with encephalitis and epilepsy is growing.<sup>14-16</sup> Our young patient presented with encephalopathy and early-onset epileptic spasms but did not have an obvious encephalitic illness. She did not have MRI findings typical of limbic encephalitis, indeed most of the children we have described with VGKC encephalitis so far had normal or non-specific acute MRI findings.<sup>15</sup> This is the first evidence for a specific autoimmunity in a patient with epileptic spasms. The clues to the possible role of autoimmunity in this case were the presence of raised CSF neopterin levels and mirrored OCBs. The presence of OCBs in CSF without corresponding serum OCB represents intrathecal synthesis of immunoglobulin-G and is highly suggestive of immune activation within the central nervous system. The presence of mirrored OCBs in both CSF and serum (as in our case) might reflect systemic synthesis of immunoglobulin-G with overflow into CSF, possibly related to blood-brain barrier dysfunction. Although mirrored OCBs might not be as diagnostically important as intrathecal OCB synthesis, mirrored OCBs are seen in some patients with acute disseminated encephalomyelitis and early NMDAR encephalitis, and might support an immune-mediated hypothesis.<sup>19</sup> The seizures in our case were refractory to conventional antiepileptic medications and partly responsive to steroids. Steroids are used in the treatment of epileptic spasms, and although the mechanism of

their action is unclear, different theories have been proposed.<sup>5</sup> It is possible that steroid therapy might have a direct immune suppression effect in some patients. Although our patient appeared to respond to steroids, the treatment was late in the course owing to the patient's location and loss to follow-up. It is tempting to speculate that early and aggressive therapy could improve outcomes in such patients. The presence of clinical and biochemical markers of immune activation in this case prompted us to search for antineuronal antibodies. Antibodies against GAD, NMDAR, LGI1, and CASPR2 were negative. VGKC-complex antibodies in this case were 201pmol/L, using a threshold of 100pmol/L as defined in adults. We have previously tested 15 healthy children for VGKC-complex antibodies (mean 18pmol/L, mean+3SD=96), suggesting the threshold of 100pmol/L is appropriate for children. Using 69 paediatric comparisons with other neurological disorders, we have previously shown that an elevated VGKC-complex antibody is rare in children.<sup>15</sup> Haberlandt et al.<sup>14</sup> have proposed the following diagnostic range for VGKC-complex antibodies in children: normal, less than 100pmol/L; low positive, 100 to 150pmol/L; positive, 150 to 400pmol/L; high positive, greater than 400pmol/L. It is unclear whether VGKC-complex antibodies are pathogenic in children. Alternatively, they might represent

a marker of immune activation and potential immune therapy responsiveness. Larger cohorts of early-onset epilepsy will help answer some of these questions. Some of the VGKC-complex antibodies found in adults with limbic encephalitis were found to be directed against proteins that are tightly complexed with VGKC such as LGI1.<sup>11,12</sup> However, this has not yet been demonstrated in children with VGKC-complex antibodies,<sup>15</sup> which suggests that these antibodies bind to either one of the VGKC-complex subunits or other proteins within the VGKC complex.

The case reported here suggests that VGKC-complex antibodies might be associated with a broader clinical spectrum than limbic encephalitis in children. Identifying these cases is important as early aggressive immune therapy might improve the outcome. Larger studies to address a possible autoimmune role in infantile epileptic encephalopathy are needed.

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#### REFERENCES

- Dulac O. Epileptic encephalopathy. *Epilepsia* 2001; **42**: 23–6.
- Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 2010; **51**: 676–85.
- Kossoff EH, Hartman AL, Rubenstein JE, Vining EP. High-dose oral prednisolone for infantile spasms: an effective and less expensive alternative to ACTH. *Epilepsy Behav* 2009; **14**: 674–6.
- Osborne JP, Lux AL, Edwards SW, et al. The underlying etiology of infantile spasms (West syndrome): information from the United Kingdom Infantile Spasms Study (UKISS) on contemporary causes and their classification. *Epilepsia* 2010; **51**: 2168–74.
- Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol* 2011; **7**: 31–40.
- Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004; **127**: 701–12.
- Pellkofer HL, Kuempfel T, Jacobson L, Vincent A, Derfuss T. Non-paraneoplastic limbic encephalitis associated with NMDAR and VGKC antibodies. *J Neurol Neurosurg Psychiatry* 2010; **81**: 1407–8.
- Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann Neurol* 2010; **67**: 470–8.
- Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 2008; **7**: 1091–8.
- Niehusmann P, Dalmau J, Rudlowski C, et al. Diagnostic value of N-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. *Arch Neurol* 2009; **66**: 458–64.
- Irani SR, Alexander S, Waters P, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 2010; **133**: 2734–48.
- Lai M, Huijbers MG, Lancaster E, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 2010; **9**: 776–85.
- Irani SR, Mitchell AW, Lang B. Faciobrachial dystonic seizures precede LGI1 antibody limbic encephalitis. *Ann Neurol* 2011; **69**: 892–900.
- Haberlandt E, Bast T, Ebner A, et al. Limbic encephalitis in children and adolescents. *Arch Dis Child* 2011; **96**: 186–91.
- Suleiman J, Brenner T, Gill D, et al. VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology* 2011; **76**: 1252–5.
- Dhamija R, Renaud DL, Pittock SJ, et al. Neuronal voltage-gated potassium channel complex autoimmunity in children. *Pediatr Neurol* 2011; **44**: 275–81.
- Dale RC, Brilot F, Fagan E, Earl J. Cerebrospinal fluid neopterin in paediatric neurology: a marker of active central nervous system inflammation. *Dev Med Child Neurol* 2009; **51**: 317–23.
- Lux AL, Edwards SW, Hancock E, et al. The United Kingdom Infantile Spasms Study comparing vigabatrin with prednisolone or tetracosactide at 14 days: a multicentre, randomised controlled trial. *Lancet* 2004; **364**: 1773–8.
- Dale RC, Brilot F. Biomarkers of inflammatory and autoimmune central nervous system disorders. *Curr Opin Pediatr* 2010; **22**: 718–25.

# VGKC antibodies in pediatric encephalitis presenting with status epilepticus



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## ABSTRACT

**Background:** Voltage-gated potassium channel antibodies (VGKC Ab) are associated with limbic encephalitis and neuromyotonia in adults. There have been no systematic investigations in children to date.

**Methods:** We looked for antibodies that are associated with CNS syndromes in adults including antibodies to VGKCs, NMDARs, glutamic acid decarboxylase (GAD), and glycine receptor (GlyR) in the stored acute serum from 10 children with unexplained encephalitis presenting with encephalopathy and status epilepticus. We also looked for antibodies to leucine-rich glioma-inactivated 1 (Lgi1) and contactin-associated protein-like 2 (Caspr2), which are now known to be tightly complexed with VGKCs in vivo. Sixty-nine pediatric controls were used for comparison.

**Results:** An elevated VGKC Ab (>100 pM) was detected in 4/10 patients with encephalitis compared to only 1/69 controls ( $p < 0.001$ ). The outcome in the 4 VGKC Ab-positive patients with encephalitis was variable including good recovery ( $n = 1$ ), cognitive impairment ( $n = 3$ ), temporal lobe epilepsy ( $n = 2$ ), and mesial temporal sclerosis ( $n = 1$ ). No other antibodies were detected, including those to Lgi1 and Caspr2.

**Conclusion:** Encephalitis associated with VGKC Ab occurs in children and presents with status epilepticus and focal epilepsy. These antibodies are not directed against Lgi1 or Caspr2.

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## GLOSSARY

**Ab** = antibody; **AERRPS** = acute encephalitis with refractory, repetitive partial seizures; **Caspr2** = contactin-associated protein-like 2; **DESC** = devastating epileptic encephalopathy in school-aged children; **GAD** = glutamic acid decarboxylase; **GlyR** = glycine receptor; **ICU** = intensive care unit; **IgG** = immunoglobulin G; **IgM** = immunoglobulin M; **LE** = limbic encephalitis; **Lgi1** = leucine-rich glioma-inactivated 1; **SE** = status epilepticus; **TLE** = temporal lobe epilepsy; **VGKC** = voltage-gated potassium channel.

Encephalitis, inflammation of the brain parenchyma, has infectious and immune-mediated etiologies.<sup>1</sup> However, in more than 50% of cases, the cause remains undefined.<sup>2</sup> Recently, there have been descriptions of autoimmune encephalitis associated with specific neuronal autoantibodies.<sup>3</sup> Autoantibodies against voltage-gated potassium channels (VGKC) are found in a proportion of adult patients with immune-responsive limbic encephalitis (LE) and neuromyotonia.<sup>4,6</sup> With the exception of a case report of a child with temporal lobe epilepsy (TLE) and elevated VGKC antibodies (Ab) 2 years following a suspected LE episode, there have been no descriptions of VGKC encephalitis in children.<sup>7</sup> VGKC Ab are measured by immunoprecipitation of VGKCs extracted from mammalian brain and labeled with 125-I- $\alpha$ -dendrotoxin that binds specifically to certain VGKC subtypes. However, recent reports suggest that many of the antibodies are directed toward other proteins, such as leucine-rich glioma-inactivated 1 (Lgi1) and contactin-associated protein-like 2 (Caspr2), which are tightly complexed with VGKCs in vivo and in tissue extracts<sup>6,8</sup> so that antibodies to these VGKC-complex antigens coimmunoprecipitate the 125-I- $\alpha$ -dendrotoxin-labeled VGKCs.<sup>6</sup> We screened for VGKC Ab by immunoprecipitation in a cohort of children with unex-

Supplemental data at  
[www.neurology.org](http://www.neurology.org)

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**Table Patients with encephalitis presenting with status epilepticus who had VGKC Ab >100 pM**

| Age, y/sex | Predominant seizure type, course                              | Other clinical features                                  | CSF microscopy and protein           | EEG   | MRI   | VGKC Ab (pM) | Outcome (length of follow-up, months)                                    |
|------------|---|--|--------------------------------------|---|---|--------------|--|
| 1/F        | Generalized Sx, seizure cluster (5 days)                      | Encephalopathy, behavioral alteration, fever             | 9 cells/mm <sup>3</sup> , 0.24 g/dL  | Left temporal slowing   | Normal <sup>a</sup>                                     | 207          | Temporal lobe epilepsy, cognitive impairment (66)                        |
| 9/F        | Secondary generalized Sx, seizure cluster (20 days)           | Encephalopathy, behavior alteration, fever               | 11 cells/mm <sup>3</sup> , 0.55 g/dL | Generalized with dominant right temporal slowing                                    | Subcortical hyperintensities (bifrontal, left parietal) | 107          | Temporal lobe epilepsy, cognitive impairment, psychiatric disorders (54) |
| 12/M       | Generalized Sx, seizure cluster (10 days)                     | Encephalopathy, cognitive and behavior alteration        | 25 cells/mm <sup>3</sup> , 0.52 g/dL | Generalized slowing   | Generalized mild cerebral edema                         | 214          | Cognitive impairment (mild) (17)   |
| 14/F       | Secondary generalized and focal Sx, seizure cluster (16 days) | Encephalopathy, cognitive and behavior alteration, fever | 6 cells/mm <sup>3</sup> , 0.21 g/dL  | Generalized slowing; right and left temporal epileptic discharges and electrical Sx | Normal  | 640          | Complete recovery (15) <sup>b</sup>                                      |

Abbreviations: Ab = antibody; Sx = seizure; VGKC = voltage-gated potassium channel.

<sup>a</sup> This patient had mesial temporal sclerosis on convalescent imaging.

<sup>b</sup> This patient was treated with IV immunoglobulin and oral steroids due to persistent cognitive impairment 6 months after acute encephalitis (see case report in appendix e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org)).

plained encephalitis presenting with status epilepticus (SE) and refractory seizures, and then asked whether they were directed against Lgi1 or Caspr2.

**METHODS** We identified 10 patients with unexplained encephalitis presenting with status epilepticus and refractory seizures between 2003 and 2009 (4 boys, mean age 7.5 years, range 1–14 years). All 10 patients had no previous seizures; 8 had no preceding neurologic abnormality and 2 had preceding mild developmental delay. All 10 patients fulfilled the case definition of encephalitis as previously described.<sup>1,2</sup> A case was defined by the presence of encephalopathy (depressed or altered level of consciousness lasting more than 24 hours, lethargy, or change in personality or behavior) with 2 or more of the following symptoms: fever, seizure, focal neurologic findings, CSF pleocytosis, or electroencephalograph or neuroimaging findings consistent with encephalitis.<sup>1,2</sup> The mean length of hospital stay for the encephalitis event was 19.7 days (median 24.5 days, range 6–36 days). Nine of the 10 patients (90%) were admitted to the intensive care unit (ICU); the mean length of ICU stay was 9 days (median 5 days, range 2–25 days). The SE was convulsive in all 10 patients, focal in 2, generalized in 5, and secondary generalized in 3. The following results were negative or normal: CSF HSV PCR (n = 8), CSF enterovirus PCR (n = 6), serology for mycoplasma pneumoniae (n = 9), enterovirus (n = 7), cytomegalovirus (n = 6), Epstein-Barr virus (n = 6), herpes simplex virus (n = 5), human herpesvirus 6 (n = 3), influenza (n = 4), and adenovirus (n = 3). The stored serum used for antineuronal antibody testing was acute serum from the first week of the encephalitis admission in all 10 patients. Sera were tested for antibodies to VGKC, NMDAR, GAD, and GlyR with methods currently in use for routine diagnosis.<sup>5,6,9–11</sup> Lgi1 and Caspr2 antibodies were tested using newly developed cell-based assays.<sup>6</sup> The project was approved by the local ethics committee and consent to test the stored serum was obtained from families. The mean length of follow-up was 22 months (median 15.5 months, range 1–66 months). Two patients had ongoing epilepsy, 5 had cognitive impairment, and 1 had died. Only 2 patients were normal on follow-up.

**Control group.** To determine the specificity of VGKC autoantibodies in children, we used 69 childhood controls including

15 healthy children (8 male, mean age 11 years, range 9–13 years), 14 with noninflammatory neurologic disorders (6 male, mean age 7.8 years, range 3–15 years), 19 with immune-mediated ataxia (11 male, mean age 6 years, range 1–11 years), 10 with encephalitis lethargica and parkinsonian features (6 male, mean age 9.4 years, range 5–15 years), and 11 with NMDAR encephalitis (2 male, mean age 6.53 years, range 1.3–13 years).<sup>12</sup> The routine laboratory cutoff for VGKC Ab is 100 pM, which is the adult healthy control mean + 3 SD. The mean VGKC Ab in our pediatric healthy control group was 18 pM (SD 26, mean + 3 SD = 96). We therefore used the established cutoff of 100 pM. The VGKC Ab findings in the encephalitis/SE group were compared with the total control group using the Fisher exact nonparametric 2 × 2 test.

**RESULTS** Four of 10 patients with encephalitis/SE had a positive VGKC Ab titer (>100 pM) compared with only 1/69 of the pediatric control group (mean 20 pM, SD 32, *p* < 0.001, 95% confidence interval). The positive control (VGKC Ab 173 pM) had NMDAR encephalitis with refractory clinical seizures. The 10 encephalitis/SE patients were all negative for antibodies against Lgi-1, Caspr2, NMDAR, GAD, and GlyR.

The clinical features of the VGKC Ab-positive patients are presented in the table (1 male, age range 1–14 years). All 4 patients were normal before the acute encephalitis, and had SE at presentation. The duration of SE was 30–60 minutes in one patient, 60 minutes–24 hours in one patient, and longer than 24 hours in 2 patients. Patients had ongoing refractory seizure clusters for 5–20 days with up to 15 seizure clusters per day (table). In addition to seizures, all patients had encephalopathy and behavioral or cognitive alteration during the acute illness. The 4 patients had a mean hospital admission of 20 days (range 7–28 days), and all required admission to intensive care for a mean of 7 days (range 2–18 days).

The CSF was abnormal in all 4 VGKC-positive patients with mild pleocytosis (n = 4) and elevated CSF protein (n = 2) (table). CSF PCR for herpes simplex virus and enterovirus was negative in all 3 patients tested. Serology for neurotropic infectious agents was negative. ANA was elevated at 1:640 in 2 patients but resolved on follow-up. One patient (case 1) had hyponatremia (Na 126 mmol/L). Brain MRI showed abnormalities in 2 (table). EEG showed slowing in all 4 patients, generalized in 2 and focal (temporal) in 2. Epileptic activity or electrical seizures were seen in one patient and were of focal (temporal) onset.

The 4 VGKC-positive patients were followed for a mean of 38 months (range 14–66). Only one made a good recovery: 2 patients had ongoing temporal lobe epilepsy, cognitive impairment, and behavioral/psychological alteration, and one had cognitive impairment only. The patient who made a good recovery (case 4 in the table) is presented in appendix e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org). Convalescent MRI showed left mesial temporal sclerosis in case 1 and persistence of the hyperintensities in case 2. Of the 6 VGKC-negative encephalitis patients who were followed up for a mean of 11 months (range 1–20), one has died, 2 have neurodevelopmental disability, and 3 are normal.

**DISCUSSION** VGKC Ab are associated with a form of LE in adults, termed VGKC Ab-associated encephalitis, which is usually seen in patients over 40 years of age. In contrast to the paraneoplastic forms of LE, this form of LE is usually nonparaneoplastic and thought to be immune responsive with a good outcome if treated adequately.<sup>4–6</sup> Our retrospective study suggests an encephalitis associated with VGKC Ab exists in children and is characterized by encephalitis with seizures and cognitive and behavioral alteration. VGKC Ab detection is performed on serum rather than CSF, as CSF levels are usually relatively low and sometimes negative. The VGKC Ab assay measures immunoglobulin G (IgG) rather than immunoglobulin M (IgM). Longitudinal studies of VGKC IgG and IgM would help understand the evolution of the autoimmune process relative to the clinical encephalitis. Although these patients had elevated VGKC Ab, they did not have antibodies to Lgi1 or Caspr2, suggesting that these pediatric patients are different from the LE associated with Lgi1 antibodies described recently in adults.<sup>6,8</sup> Further analysis of their sera is in progress to determine whether their antibodies are directed against the VGKC subunits or other proteins in the VGKC complex. Our control group suggest that elevated

VGKC Ab are rare in children: the only control with an elevated VGKC Ab titer had a clinical phenotype of NMDAR encephalitis and had both NMDAR and VGKC Ab.<sup>12</sup> The finding of more than one antibody associated with autoimmune encephalitis has been recently reported in an adult patient.<sup>13</sup>

The encephalitis in these patients was previously unexplained and CSF testing for common infectious agents and serologic investigations were negative. As the diagnosis of VGKC Ab-associated encephalitis was made retrospectively using stored acute serum, none of our patients received immunotherapy during the acute illness. The 4 VGKC Ab patients have generally done poorly: 2 patients have ongoing TLE and one has cognitive impairment. Prospective studies including longitudinal VGKC Ab measurement before and after immune therapy are required to improve our understanding of VGKC encephalitis in children.

The initial clinical course of case 4 was reminiscent of devastating epileptic encephalopathy in school-aged children (DESC). DESC is a pseudoencephalitis syndrome of unknown cause that affects previously well children, causes refractory seizures and encephalopathy, and has severe morbidity and mortality.<sup>14</sup> The cases are also reminiscent of acute encephalitis with refractory, repetitive partial seizures (AERRPS), an unexplained encephalitis associated with seizures which is described mainly in Japanese patients.<sup>15</sup> It is possible that autoimmune mechanisms may be important in some cases of DESC and AERRPS, and this requires investigation.

One case with VGKC Ab encephalitis (case 1) had a normal MRI during her acute encephalitis while follow-up imaging showed left mesial temporal sclerosis. This patient has ongoing TLE; the evolution from LE to TLE and mesial temporal sclerosis has been described in adults.<sup>16</sup> Elevated VGKC Ab were recently described in a 13-year-old girl with TLE and hippocampal sclerosis 2 years after a suspected LE.<sup>7</sup>

The role of VGKC Ab requires further investigation in larger cohorts of pediatric encephalitis, and children with TLE and mesial temporal sclerosis.

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#### DISCLOSURE

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ica; has received funding for travel and a speaker honorarium from Baxter International Inc.; serves as an Associate Editor for *Brain*; receives royalties from the publication of *Clinical Neuroimmunology* (Blackwell Publishing, 2005); receives research support from the European Union, NIHR Biomedical Research Centre Oxford, and Sir Halley Stewart Trust; and has received Musk antibody royalties and consulting fees from Athena Diagnostics, Inc., and Musk antibody royalties from RSR Ltd., Cardiff, UK. The University of Oxford, where A.V. is based, receives royalties and payments for antibody assays in neurologic diseases. Dr. Lang reports no disclosures. Dr. Dale serves on a scientific advisory board for the Brisbane Children's Hospital; receives publishing royalties for *Autoimmune and Inflammatory Disorders of the Nervous System in Children* (MacKeith Press, 2009); has received speaker honoraria from Biogen Idec; and receives research support from Pfizer Inc., the Tourette Syndrome Association, USA, the Brain Foundation Australia, and the Multiple Sclerosis Foundation Australia, and the University of Sydney.

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## REFERENCES

1. Granerod J, Cunningham R, Zuckerman M, et al. Causality in acute encephalitis: defining aetiologies. *Epidemiol Infect* 2010;138:783–800.
2. Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. *Clin Infect Dis* 2003;36:731–742.
3. Vincent A, Irani SR, Lang B. The growing recognition of immunotherapy-responsive seizure disorders with autoantibodies to specific neuronal proteins. *Curr Opin Neurol* 2010;23:144–150.
4. Thieben MJ, Lennon VA, Boeve BF, Aksamit AJ, Keegan M, Vernino S. Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. *Neurology* 2004;62:1177–1182.
5. Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004;127:701–712.
6. Irani SR, Alexander S, Waters P, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 2010;133:2734–2748.
7. Kroll-Seeger J, Bien CG, Huppertz HJ. Non-paraneoplastic limbic encephalitis associated with antibodies to potassium channels leading to bilateral hippocampal sclerosis in a pre-pubertal girl. *Epileptic Disord* 2009;11:54–59.
8. Lai M, Huijbers MG, Lancaster E, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 2010;9:776–785.
9. McKnight K, Jiang Y, Hart Y, et al. Serum antibodies in epilepsy and seizure-associated disorders. *Neurology* 2005;65:1730–1736.
10. Hutchinson M, Waters P, McHugh J, et al. Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. *Neurology* 2008;71:1291–1292.
11. Irani SR, Bera K, Waters P, et al. N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* 2010;133:1655–1667.
12. Dale RC, Irani SR, Brilot F, et al. N-methyl-D-aspartate receptor antibodies in pediatric dyskinetic encephalitis lethargica. *Ann Neurol* 2009;66:704–709.
13. Pellkofer HL, Kuempfel T, Jacobson L, Vincent A, Derfuss T. Non-paraneoplastic limbic encephalitis associated with NMDAR and VGKC antibodies. *J Neurol Neurosurg Psychiatry* 2010;81:1407–1408.
14. Mikaeloff Y, Jambaque I, Hertz-Pannier L, et al. Devastating epileptic encephalopathy in school-aged children (DESC): a pseudo encephalitis. *Epilepsy Res* 2006;69:67–79.
15. Sakuma H. Acute encephalitis with refractory, repetitive partial seizures. *Brain Dev* 2009;31:510–514.
16. Bien CG, Elger CE, Bien CG, Elger CE. Limbic encephalitis: a cause of temporal lobe epilepsy with onset in adult life. *Epilepsy Behav* 2007;10:529–538.

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## 7. REFERENCES

- Akman CI, Patterson MC, Rubinstein A, Herzog R (2009) Limbic encephalitis associated with anti-GAD antibody and common variable immune deficiency. *Developmental Medicine and Child Neurology* 51:563-567.
- Andrade D, Tai P, Dalmau J, Wennberg R (2011a) Tonic seizure: a diagnostic clue of anti-LGI1 encephalitis? Reply. *Neurology* 77:2141-2143.
- Andrade DM, Tai P, Dalmau J, Wennberg R (2011b) Tonic seizure: a diagnostic clue of anti-LGI1 encephalitis? *Neurology* 76:1355-1357.
- Archelos JJ, Hartung H-P (2000) Pathogenetic role of autoantibodies in neurological diseases. *Trends in Neurosciences* 23:317-327.
- Armangue T, Titulaer MJ, Malaga I, Bataller L, Gabilondo I, Graus F, Dalmau J, Spanish Anti Nm-DA (2013) Pediatric Anti-N-methyl-D-Aspartate Receptor Encephalitis-Clinical Analysis and Novel Findings in a Series of 20 Patients. *Journal of Pediatrics* 162:850-+.
- Bakheit AMO, Kennedy PGE, Behan PO (1990) Paraneoplastic limbic encephalitis - clinicopathological correlations. *Journal of Neurology Neurosurgery and Psychiatry* 53:1084-1088.
- Bancaud J, Henriksen O, Rubiodonnadieu F, Seino M, Dreifuss FE, Penry JK (1981) PROPOSAL FOR REVISED CLINICAL AND ELECTROENCEPHALOGRAPHIC CLASSIFICATION OF EPILEPTIC SEIZURES. *Epilepsia* 22:489-501.
- Barajas RF, Collins DE, Cha S, Geschwind MD (2010) Adult-onset drug-refractory seizure disorder associated with anti-voltage-gated potassium-channel antibody. *Epilepsia* 51:473-477.
- Barber PA, Anderson NE, Vincent A (2000) Morvan's syndrome associated with voltage-gated K+ channel antibodies. *Neurology* 54:771-772.
- Beghi E, Carpio A, Forsgren L, Hesdorffer DC, Malmgren K, Sander JW, Tomson T, Hauser WA (2010) Recommendation for a definition of acute symptomatic seizure. *Epilepsia* 51:671-675.
- Beleza P (2012) Acute Symptomatic Seizures A Clinically Oriented Review. *Neurologist* 18:109-119.
- Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, Boas WV, Engel J, French J, Glauser TA, Mathern GW, Moshe SL, Nordli D, Plouin P, Scheffer IE (2010) Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 51:676-685.
- Berg AT, Scheffer IE (2011) New concepts in classification of the epilepsies: Entering the 21st century. *Epilepsia* 52:1058-1062.
- Berg AT, Shinnar S, Hauser WA, Alemany M, Shapiro ED, Salomon ME, Crain EF (1992) A PROSPECTIVE-STUDY OF RECURRENT FEBRILE SEIZURES. *New England Journal of Medicine* 327:1122-1127.
- Berkovic SF, Mulley JC, Scheffer IE, Petrou S (2006) Human epilepsies: interaction of genetic and acquired factors. *Trends in Neurosciences* 29:391-397.
- Bhagavati S, Maccabee PJ, Chari G (2007) Is cerebral involvement an occasional feature of muscle-specific kinase antibody-positive syndrome? *European Journal of Neurology* 14:E21-E22.
- Bien CG, Elger CE, Bien CG, Elger CE (2007a) Limbic encephalitis: a cause of temporal lobe epilepsy with onset in adult life. *Epilepsy & Behavior* 10:529-538.
- Bien CG, Urbach H, Schramm J, Soeder BM, Becker AJ, Voltz R, Vincent A, Elger CE (2007b) Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology* 69:1236-1244.
- Bien CG, Vincent A, Barnett MH, Becker AJ, Blumcke I, Graus F, Jellinger KA, Reuss DE, Ribalta T, Schlegel J, Sutton I, Lassmann H, Bauer J (2012) Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* 135:1622-1638.
- Bliss TVP, Collingridge GL (1993) A SYNAPTIC MODEL OF MEMORY - LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. *Nature* 361:31-39.



- Blume WT, Luders HO, Mizrahi E, Tassinari C, van Emde Boas W, Engel J, Jr. (2001) Glossary of descriptive terminology for ictal semiology: Report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 42:1212-1218.
- Boronat A, Sabater L, Saiz A, Dalmau J, Graus F (2011) GABA(B) receptor antibodies in limbic encephalitis and anti-GAD-associated neurologic disorders. *Neurology* 76:795-800.
- Brenner T, Sills GJ, Hart Y, Howell S, Waters P, Brodie MJ, Vincent A, Lang B (2013) Prevalence of neurologic autoantibodies in cohorts of patients with new and established epilepsy. *Epilepsia* n/a-n/a.
- Brigo F, Bongiovanni LG, Cerini R, Manganotti P, Storti M, Fiaschi A (2011) Infliximab-related seizures: a first case study. *Epileptic Disorders* 13:214-217.
- Brown P, Marsden CD (1999) The stiff man and stiff man plus syndromes. *Journal of Neurology* 246:648-652.
- Buckley C, Oger J, Clover L, Tuzun E, Carpenter K, Jackson M, Vincent A (2001) Potassium channel antibodies in two patients with reversible limbic encephalitis. *Annals of Neurology* 50:73-78.
- Camfield CS, Camfield PR, Gordon K, Wirrell E, Dooley JM (1996) Incidence of epilepsy in childhood and adolescence: A population-based study in Nova Scotia from 1977 to 1985. *Epilepsia* 37:19-23.
- Castillo P, Woodruff B, Caselli R, Vernino S, Lucchinetti C, Swanson J, Noseworthy J, Aksamit A, Carter J, Sirven J, Hunder G, Fatourechi V, Mokri B, Drubach D, Pittock S, Lennon V, Boeve B (2006) Steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Archives of Neurology* 63:197-202.
- Castillo PR, Boeve BF, Caselli RJ, Vernino SA, Lucchinetti C, Swanson JW, Noseworthy JH, Aksamit AJ, Flemming KD, Hunder GG, Fatourechi V, Mokri B, Lennon VA (2002) Steroid-responsive encephalopathy associated with thyroid autoimmunity: Clinical and laboratory findings. *Neurology* 58:A248-A248.
- Chin RFM, Neville BGR, Peckham C, Bedford H, Wade A, Scott RC, Grp NC (2006) Incidence, cause, and short-term outcome of convulsive status epilepticus in childhood: prospective population-based study. *Lancet* 368:222-229.
- Chin RFM, Verhulst L, Neville BGR, Peters MJ, Scott RC (2004) Inappropriate emergency management of status epilepticus in children contributes to need for intensive care. *Journal of Neurology Neurosurgery and Psychiatry* 75:1584-1588.
- Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. *Neuropharmacology* 56:2-5.
- Commission On E, Prognosis International League A, Epilepsy (1993) Guidelines for epidemiologic studies on epilepsy. *Epilepsia* 34:592-596.
- Dale RC, Brilot F, Fagan E, Earl J (2009a) Cerebrospinal fluid neopterin in paediatric neurology: a marker of active central nervous system inflammation. *Dev Med Child Neurol* 51:317-323.
- Dale RC, Irani SR, Brilot F, Pillai S, Webster R, Gill D, Lang B, Vincent A (2009b) N-Methyl-D-Aspartate Receptor Antibodies in Pediatric Dyskinetic Encephalitis Lethargica. *Annals of Neurology* 66:704-709.
- Dale RC, Merheb V, Pillai S, Wang D, Cantrill L, Murphy TK, Ben-Pazi H, Varadkar S, Aumann TD, Horne MK, Church AJ, Fath T, Brilot F (2012) Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain*.
- Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng XY, Lai MZ, Dessain SK, Rosenfeld MR, Balice-Gordon R, Lynch DR (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurology* 7:1091-1098.
- Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R (2011) Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* 10:63-74.
- Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR (2007) Paraneoplastic anti-N-

- methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Annals of Neurology* 61:25-36.
- Damasio J, Leite MI, Coutinho E, Waters P, Woodhall M, Santos MA, Carrilho I, Vincent A (2013) Progressive encephalomyelitis with rigidity and myoclonus: the first pediatric case with glycine receptor antibodies. *JAMA Neurol* 70:498-501.
- Dhamija R, Renaud DL, Pittock SJ, McKeon A, Lachance DH, Nickels KC, Wirrell EC, Kuntz NL, King MD, Lennon VA (2011) Neuronal Voltage-Gated Potassium Channel Complex Autoimmunity in Children. *Pediatric Neurology* 44:275-281.
- Dulac O (2001) Epileptic encephalopathy. *Epilepsia* 42:23-26.
- Engel J (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* 47:1558-1568.
- Eriksson KJ, Koivikko MJ (1997) Prevalence, classification, and severity of epilepsy and epileptic syndromes in children. *Epilepsia* 38:1275-1282.
- Errichiello L, Perruolo G, Pascarella A, Formisano P, Minetti C, Striano S, Zara F, Striano P (2009) Autoantibodies to glutamic acid decarboxylase (GAD) in focal and generalized epilepsy: A study on 233 patients. *Journal of Neuroimmunology* 211:120-123.
- Finke C, Pruess H, Scheel M, Ostendorf F, Harms L, Borowski K, Wandinger K-P, Ploner CJ (2012) Anti-NMDA receptor antibodies in a case of MELAS syndrome. *Journal of Neurology* 259:582-584.
- Florance NR, Davis RL, Lam C, Szperka C, Zhou L, Ahmad S, Campen CJ, Moss H, Peter N, Gleichman AJ, Glaser CA, Lynch DR, Rosenfeld MR, Dalmau J (2009) Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 66:11-18.
- Fukata Y, Lovero KL, Iwanaga T, Watanabe A, Yokoi N, Tabuchi K, Shigemoto R, Nicoll RA, Fukata M (2010) Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proceedings of the National Academy of Sciences of the United States of America* 107:3799-3804.
- Gabilondo I, Saiz A, Galan L, Gonzalez V, Jadraque R, Sabater L, Sans A, Sempere A, Vela A, Villalobos F, Vinals M, Villoslada P, Graus F (2011) Analysis of relapses in anti-NMDAR encephalitis. *Neurology* 77:996-999.
- Gable MS, Gavali S, Radner A, Tilley DH, Lee B, Dwyer L, Collins A, Dengel A, Dalmau J, Glaser CA (2009) Anti-NMDA receptor encephalitis: report of ten cases and comparison with viral encephalitis. *European Journal of Clinical Microbiology & Infectious Diseases* 28:1421-1429.
- Gastaut H (1970) Clinical and Electroencephalographical Classification of Epileptic Seizures. *Epilepsia* 11:102-&.
- Gastaut H (1983) Classification of status epilepticus. *Advances in neurology* 34:15-35.
- Gastaut H, Caveness WF, Landolt H, Dehaas AML, McNaughton FL, Magnus O, Merlis JK, Pond DA, Radermecker J, Vanleeuwen WS (1964) A Proposed International Classification of Epileptic Seizures. *Epilepsia* 5:297-306.
- Giometto B, Nicolao P, Macucci M, Tavalato B, Foxon R, Bottazzo GF (1998) Temporal-lobe epilepsy associated with glutamic-acid-decarboxylase autoantibodies. *Lancet* 352:457-457.
- Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, Fischer M, Cossen CK, Anderson LJ (2003) In search of encephalitis etiologies: Diagnostic challenges in the California Encephalitis Project, 1998-2000. *Clinical Infectious Diseases* 36:731-742.
- Go CY, Mackay MT, Weiss SK, Stephens D, Adams-Webber T, Ashwal S, Snead OC, III (2012) Evidence-based guideline update: Medical treatment of infantile spasms Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 78:1974-1980.
- Gold M, Pul R, Bach J-P, Stangel M, Dodel R (2012) Pathogenic and physiological autoantibodies in the central nervous system. *Immunological Reviews* 248:68-86.
- Granerod J, Ambrose HE, Davies NWS, Clewley JP, Walsh AL, Morgan D, Cunningham R, Zuckerman M, Mutton KJ, Solomon T, Ward KN, Lunn MPT, Irani SR, Vincent A, Brown DWG, Crowcroft

- NS, HPA UKHPA (2010) Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infectious Diseases* 10:835-844.
- Graus F, Saiz A, Dalmau J (2010) Antibodies and neuronal autoimmune disorders of the CNS. *J Neurol* 257:509-517.
- Gultekin SH, Rosenfeld MR, Voltz R, Eichen J, Posner JB, Dalmau J (2000) Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumour association in 50 patients. *Brain* 123:1481-1494.
- Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stuhmer W, Wang XL (2005) International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacological Reviews* 57:473-508.
- Haberlandt E, Bast T, Ebner A, Holthausen H, Kluger G, Kravljanc R, Kroll-Seger J, Kurlemann G, Makowski C, Rostasy K, Tuschen-Hofstatter E, Weber G, Vincent A, Bien CG (2011) Limbic encephalitis in children and adolescents. *Arch Dis Child* 96:186-191.
- Hacohen Y WS, Waters P, Agrawal S, Carr L, Cross H, De Sousa C, DeVile C, Fallon P, Gupta R, Hedderly T, Hughes E, Kerr T, Lascelles K, Lin J-P, Philip S, Pohl K, Prabahkar P, Smith M, Williams R, Clarke A, Hemingway C, Wassmer E, Vincent A, Lim M. (2013) Paediatric autoimmune encephalopathies: clinical features, laboratory investigations and outcomes in patients with or without antibodies to known central nervous system (CNS) autoantigens. *J Neurol Neurosurg Psychiatr* (in press) PMID: 23175854.
- Hart IK, Waters C, Vincent A, Newland C, Beeson D, Pongs O, Morris C, NewsomDavis J (1997) Autoantibodies detected to expressed K<sup>+</sup> channels are implicated in neuromyotonia. *Annals of Neurology* 41:238-246.
- Hartley LM, Ng SY, Dale RC, Church AJ, Martinez A, de Sousa C (2002) Immune mediated chorea encephalopathy syndrome in childhood. *Developmental Medicine and Child Neurology* 44:273-277.
- Helbig I, Scheffer IE, Mulley JC, Berkovic SF (2008) Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurology* 7:231-245.
- Holland KD, Hallinan BE (2010) What causes epileptic encephalopathy in infancy? The answer may lie in our genes. *Neurology* 75:1132-1133.
- Honorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, Maestre J, Fabien N, Vighetto A, Casamitjana R, Thivolet C, Tavolato B, Antoine JC, Trouillas P, Graus F (2001) Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies - Study of 14 patients. *Archives of Neurology* 58:225-230.
- Howell KB, Katanyuwong K, Mackay MT, Bailey CA, Scheffer IE, Freeman JL, Berkovic SF, Harvey AS (2012) Long-term follow-up of febrile infection-related epilepsy syndrome. *Epilepsia* 53:101-110.
- Hutchinson M, Waters P, McHugh J, Gorman G, O'Riordan S, Connolly S, Hager H, Yu P, Becker CM, Vincent A (2008) Progressive encephalomyelitis, rigidity, and myoclonus: A novel glycine receptor antibody. *Neurology* 71:1291-1292.
- Illingworth MA, Hanrahan D, Anderson CE, O'Kane K, Anderson J, Casey M, de Sousa C, Cross JH, Wright S, Dale RC, Vincent A, Kurian MA (2011) Elevated VGKC-complex antibodies in a boy with fever-induced refractory epileptic encephalopathy in school-age children (FIRES). *Developmental Medicine and Child Neurology* 53:1053-1057.
- Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, Peles E, Buckley C, Lang B, Vincent A (2010a) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 133:2734-2748.
- Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi MS, Friese MA, Galea I, Kullmann DM, Beeson D, Lang B, Bien CG, Vincent A (2010b) N-methyl-D-aspartate antibody encephalitis: temporal

- progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* 133:1655-1667.
- Irani SR, Bien CG, Lang B (2011a) Autoimmune epilepsies. *Current Opinion in Neurology* 24:146-153.
- Irani SR, Buckley C, Vincent A, Cockerell OC, Rudge P, Johnson MR, Smith S (2008) Immunotherapy-Responsive Seizure-Like Episodes with Potassium Channel Antibodies. *Neurology* 71:1647-1648.
- Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, Schott JM, Armstrong RJE, S. Zagami A, Bleasel A, Somerville ER, Smith SMJ, Vincent A (2011b) Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Annals of Neurology* 69:892-900.
- Irani SR, Schott JM, Vincent A, Smith SJM (2011c) Tonic seizure: a diagnostic clue of anti-LGI1 encephalitis? *Neurology* 77:2140-2141.
- Irani SR, Vincent A (2011) NMDA Receptor Antibody Encephalitis. *Current Neurology and Neuroscience Reports* 11:298-304.
- Ismail FY, Kossoff EH (2011) AERRPS, DESC, NORSE, FIRES: Multi-labeling or distinct epileptic entities? *Epilepsia* 52:E185-E189.
- Jarius S, Hoffmann L, Clover L, Vincent A, Voltz R (2008) CSF findings in patients with voltage gated potassium channel antibody associated limbic encephalitis. *Journal of the Neurological Sciences* 268:74-77.
- Kossoff EH, Hartman AL, Rubenstein JE, Vining EP (2009) High-dose oral prednisolone for infantile spasms: an effective and less expensive alternative to ACTH. *Epilepsy Behav* 14:674-676.
- Kramer U, Chi CS, Lin KL, Specchio N, Sahin M, Olson H, Nabbout R, Kluger G, Lin JJ, van Baalen A (2011) Febrile infection-related epilepsy syndrome (FIRES): Pathogenesis, treatment, and outcome A multicenter study on 77 children. *Epilepsia* 52:1956-1965.
- Kroll-Seger J, Bien CG, Huppertz HJ (2009) Non-paraneoplastic limbic encephalitis associated with antibodies to potassium channels leading to bilateral hippocampal sclerosis in a pre-pubertal girl. *Epileptic Disorders* 11:54-59.
- Kullmann DM, Waxman SG (2010) Neurological channelopathies: new insights into disease mechanisms and ion channel function. *Journal of Physiology-London* 588:1823-1827.
- Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Hauser WA, Mathern G, Moshe SL, Perucca E, Wiebe S, French J (2010) Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51:1069-1077.
- Kwan P, Brodie MJ (2000) Early identification of refractory epilepsy. *New England Journal of Medicine* 342:314-319.
- Kwan P, Sills GJ, Kelly K, Butler E, Brodie MJ (2000) Glutamic acid decarboxylase autoantibodies in controlled and uncontrolled epilepsy: a pilot study. *Epilepsy Research* 42:191-195.
- Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, Cowell JK, Dalmau J (2010) Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 9:776-785.
- Lai MZ, Hughes EG, Peng XY, Zhou L, Gleichman AJ, Shu H, Mata S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J (2009) AMPA Receptor Antibodies in Limbic Encephalitis Alter Synaptic Receptor Location. *Annals of Neurology* 65:424-434.
- Lancaster E, Dalmau J (2012) Neuronal autoantigens-pathogenesis, associated disorders and antibody testing. *Nature Reviews Neurology* 8:380-390.
- Lancaster E, Huijbers MGM, Bar V, Boronat A, Wong A, Martinez-Hernandez E, Wilson C, Jacobs D, Lai M, Walker RW, Graus F, Bataller L, Illa I, Markx S, Strauss KA, Peles E, Scherer SS, Dalmau J (2011a) Investigations of Caspr2, an Autoantigen of Encephalitis and Neuromyotonia. *Annals of Neurology* 69:303-311.
- Lancaster E, Lai MZ, Peng XY, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J

- (2010) Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: Case series and characterisation of the antigen. *Lancet Neurology* 9:67-76.
- Lancaster E, Martinez-Hernandez E, Dalmau J (2011b) Encephalitis and antibodies to synaptic and neuronal cell surface proteins. *Neurology* 77:179-189.
- Lee EK, Maselli RA, Ellis WG, Agius MA (1998) Morvan's fibrillary chorea: a paraneoplastic manifestation of thymoma. *Journal of Neurology Neurosurgery and Psychiatry* 65:857-862.
- Leite MI, Waters P, Carvajal A, Woodhall M, Vincent A (2012) Expanding the Phenotypic Spectrum of Progressive Encephalomyelitis with Rigidity and Myoclonus Syndrome (PERMS) Associated with Glycine Receptor Antibody: Clinical and Laboratory Study of 20 Patients. *Journal of Neurology Neurosurgery and Psychiatry* 83.
- Librizzi L, Noe F, Vezzani A, de Curtis M, Ravizza T (2012) Seizure-Induced Brain-Borne Inflammation Sustains Seizure Recurrence and Blood-Brain Barrier Damage. *Annals of Neurology* 72:82-90.
- Liguori R, Vincent A, Clover L, Avoni P, Plazzi G, Cortelli P, Baruzzi A, Carey T, Gambetti P, Lugaresi E, Montagna P (2001) Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. *Brain* 124:2417-2426.
- Loukaides P, Schiza N, Pettingill P, Palazis L, Vounou E, Vincent A, Kleopa KA (2012) Morvan's syndrome associated with antibodies to multiple voltage-gated potassium channel complex. *Journal of the Neurological Sciences* 312:52-56.
- Lynch DR, Anegawa NJ, Verdoorn T, Pritchett DB (1994) N-METHYL-D-ASPARTATE RECEPTORS - DIFFERENT SUBUNIT REQUIREMENTS FOR BINDING OF GLUTAMATE ANTAGONISTS, GLYCINE ANTAGONISTS, AND CHANNEL-BLOCKING AGENTS. *Molecular Pharmacology* 45:540-545.
- Majoie HJ, de Baets M, Renier W, Lang B, Vincent A (2006) Antibodies to voltage-gated potassium and calcium channels in epilepsy. *Epilepsy Res* 71:135-141.
- Makatsori M, Manson AL, Verma N, Leandro M, Gurugama P, Longhurst H, Grigoriadou S, Buckland M, Kiani-Alikhan S, Hanson S, Ibrahim MA, Chee R, Kanfer E, Grimbacher B, Seneviratne SL (2012) POST-RITUXIMAB PROLONGED HYPOGAMMAGLOBULINEMIA REQUIRING INTRAVENOUS IMMUNOGLOBULIN REPLACEMENT THERAPY (IVIG). *Journal of Clinical Immunology* 32:283-283.
- Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG (2010) Antibodies to Glutamic Acid Decarboxylase Define a Form of Limbic Encephalitis. *Annals of Neurology* 67:470-478.
- Manford M, Hart YM, Sander J, Shorvon SD (1992) THE NATIONAL GENERAL-PRACTICE STUDY OF EPILEPSY - THE SYNDROMIC CLASSIFICATION OF THE INTERNATIONAL-LEAGUE-AGAINST-EPILEPSY APPLIED TO EPILEPSY IN A GENERAL-POPULATION. *Archives of Neurology* 49:801-808.
- Martinelli P, Pazzaglia P, Montagna P, Coccagna G, Rizzuto N, Simonati S, Lugaresi E (1978) Stiff-Man Syndrome Associated with Nocturnal Myoclonus and Epilepsy. *Journal of Neurology Neurosurgery and Psychiatry* 41:458-462.
- Mas N, Saiz A, Leite MI, Waters P, Baron M, Castano D, Sabater L, Vincent A, Graus F (2011) Antiglycine-receptor encephalomyelitis with rigidity. *Journal of Neurology Neurosurgery and Psychiatry* 82:1399-1401.
- Mastrangelo M, Leuzzi V (2012) Genes of Early-Onset Epileptic Encephalopathies: From Genotype to Phenotype. *Pediatric Neurology* 46:24-31.
- McKeon A, Martinez-Hernandez E, Lancaster E, Matsumoto JY, Harvey RJ, McEvoy KM, Pittock SJ, Lennon VA, Dalmau J (2013) Glycine receptor autoimmune spectrum with stiff-man syndrome phenotype. *JAMA neurology* 70:44-50.
- McKnight K, Jiang Y, Hart Y, Cavey A, Wroe S, Blank M, Shoenfeld Y, Vincent A, Palace J, Lang B (2005) Serum antibodies in epilepsy and seizure-associated disorders.[see comment]. *Neurology* 65:1730-1736.
- Meinck HM, Thompson PD (2002) Stiff man syndrome and related conditions. *Movement Disorders* 17:853-866.

- Mikaeloff Y, Jambaque I, Hertz-Pannier L, Zamfirescu A, Adamsbaum C, Plouin P, Dulac O, Chiron C (2006) Devastating epileptic encephalopathy in school-aged children (DESC): a pseudo encephalitis. *Epilepsy Research* 69:67-79.
- Milh M, Villeneuve N, Chapon F, Gavaret M, Girard N, Mancini J, Chabrol B, Boucraut J (2011) New onset refractory convulsive status epilepticus associated with serum neuropil auto-antibodies in a school aged child. *Brain & Development* 33:687-691.
- Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, Saenz A, Poza JJ, Galan J, Gesk S, Sarafidou T, Mautner VF, Binelli S, Staub E, Hinzmann B, French L, Prud'homme JF, Passarelli D, Scannapieco P, Tassinari CA, Avanzini G, Marti-Masso JF, Kluwe L, Deloukas P, Moschonas NK, Michelucci R, Siebert R, Nobile C, Perez-Tur J, de Munain AL (2002) Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Human Molecular Genetics* 11:1119-1127.
- Nabbout R, Mazzuca M, Hubert P, Peudennier S, Allaire C, Flurin V, Aberastury M, Silva W, Dulac O (2010) Efficacy of ketogenic diet in severe refractory status epilepticus initiating fever induced refractory epileptic encephalopathy in school age children (FIRES). *Epilepsia* 51:2033-2037.
- Nabbout R, Vezzani A, Dulac O, Chiron C (2011) Acute encephalopathy with inflammation-mediated status epilepticus. *Lancet Neurology* 10:99-108.
- Newsom-Davis J (1997) Autoimmune neuromyotonia (Isaacs' syndrome): An antibody-mediated potassium channelopathy. In: *Frontiers of Neurology: A Symposium in Honor of Fred Plum*, vol. 835 (Reis, D. J. and Posner, J. B., eds), pp 111-119 New York: New York Acad Sciences.
- Newsom-Davis J, Mills KR (1993) IMMUNOLOGICAL ASSOCIATIONS OF ACQUIRED NEUROMYOTONIA (ISAACS SYNDROME) - REPORT OF 5 CASES AND LITERATURE-REVIEW. *Brain* 116:453-469.
- Niehusmann P, Dalmau J, Rudlowski C, Vincent A, Elger CE, Rossi JE, Bien CG (2009) Diagnostic Value of N-methyl-D-aspartate Receptor Antibodies in Women With New-Onset Epilepsy. *Archives of Neurology* 66:458-464.
- Nociti V, Frisullo G, Tartaglione T, Patanella AK, Iorio R, Tonali PA, Batocchi AP (2010) Refractory generalized seizures and cerebellar ataxia associated with anti-GAD antibodies responsive to immunosuppressive treatment. *European Journal of Neurology* 17:E5-E5.
- Osborne JP, Lux AL, Edwards SW, Hancock E, Johnson AL, Kennedy CR, Newton RW, Verity CM, O'Callaghan FJ The underlying etiology of infantile spasms (West syndrome): information from the United Kingdom Infantile Spasms Study (UKISS) on contemporary causes and their classification. *Epilepsia* 51:2168-2174.
- Palace J, Lang B (2000) Epilepsy: an autoimmune disease? *Journal of Neurology Neurosurgery and Psychiatry* 69:711-714.
- Palmer CA, Geyer JD, Keating JM, Gilliam F, Kuzniecky RI, Morawetz RB, Bebin EM (1999) Rasmussen's encephalitis with concomitant cortical dysplasia: The role of GluR3. *Epilepsia* 40:242-247.
- Panayiotopoulos CP (2011) The new ILAE report on terminology and concepts for organization of epileptic seizures: A clinician's critical view and contribution. *Epilepsia* 52:2155-2160.
- Panayiotopoulos CP (2012) The new ILAE report on terminology and concepts for the organization of epilepsies: Critical review and contribution. *Epilepsia* 53:399-404.
- Pearl PL (2009) New treatment paradigms in neonatal metabolic epilepsies. *Journal of Inherited Metabolic Disease* 32:204-213.
- Pellkofer HL, Kuempfel T, Jacobson L, Vincent A, Derfuss T (2010) Non-paraneoplastic limbic encephalitis associated with NMDAR and VGKC antibodies. *Journal of Neurology Neurosurgery and Psychiatry* 81:1407-1408.
- Pellock JM (2004) Understanding co-morbidities affecting children with epilepsy. *Neurology* 62:S17-S23.

- Peltola J, Kulmala P, Isojarvi J, Saiz A, Latvala K, Palmio J, Savola K, Knip M, Keranen T, Graus F (2000) Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. *Neurology* 55:46-50.
- Pittock SJ, Lucchinetti CF, Parisi JE, Benarroch EE, Mokri B, Stephan CL, Kim KK, Killmann MW, Lennon VA (2005) Amphiphysin autoimmunity: Paraneoplastic accompaniments. *Annals of Neurology* 58:96-107.
- Quek AML, Britton JW, McKeon A, So E, Lennon VA, Shin C, Klein CJ, Watson RE, Kotsenas AL, Lagerlund TD, Cascino GD, Worrell GA, Wirrell EC, Nickels KC, Aksamit AJ, Noe KH, Pittock SJ (2012) Autoimmune Epilepsy Clinical Characteristics and Response to Immunotherapy. *Archives of Neurology* 69:582-593.
- Reid JM, Foley P, Willison HJ (2009) Voltage-gated Potassium Channel-associated Limbic Encephalitis in the West of Scotland: Case Reports and Literature Review. *Scottish Medical Journal* 54:27-31.
- Roger J, Dreifuss FE, Martinez-Lage M, Munari C, Porter RJ, Seino M, Wolf P (1989) PROPOSAL FOR REVISED CLASSIFICATION OF EPILEPSIES AND EPILEPTIC SYNDROMES. *Epilepsia* 30:389-399.
- Rogers SW, Andrews PI, Gahring LC, Whisenand T, Cauley K, Crain B, Hughes TE, Heinemann SF, McNamara JO (1994) AUTOANTIBODIES TO GLUTAMATE-RECEPTOR GLUR3 IN RASMUSSENS ENCEPHALITIS. *Science* 265:648-651.
- Rosenfeld MR, Titulaer MJ, Dalmau J (2012) Paraneoplastic syndromes and autoimmune encephalitis: Five new things. *Neurol Clin Pract* 2:215-223.
- Saiz A, Blanco Y, Sabater L, Gonzalez F, Bataller L, Casamitjana R, Ramio-Torrenta L, Graus F (2008) Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain* 131:2553-2563.
- Sakuma H (2009) Acute encephalitis with refractory, repetitive partial seizures. *Brain & Development* 31:510-514.
- Sakuma H, Awaya Y, Shiomi M, Yamanouchi H, Takahashi Y, Saito Y, Sugai K, Sasaki M (2010) Acute encephalitis with refractory, repetitive partial seizures (AERRPS): A peculiar form of childhood encephalitis. *Acta Neurologica Scandinavica* 121:251-256.
- Schauble B, Castillo PR, Boeve BF, Westmoreland BF (2003) EEG findings in steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Clinical Neurophysiology* 114:32-37.
- Schmitt SE, Pargeon K, Frechette ES, Hirsch LJ, Dalmau J, Friedman D (2012) Extreme delta brush A unique EEG pattern in adults with anti-NMDA receptor encephalitis. *Neurology* 79:1094-1100.
- Schober E, Otto KP, Dost A, Jorch N, Holl R, German Austrian DPVI, Diab BCN (2012) Association of Epilepsy and Type 1 Diabetes Mellitus in Children and Adolescents: Is There an Increased Risk for Diabetic Ketoacidosis? *Journal of Pediatrics* 160:662-666.
- Schulte U, Thumfart JO, Klocker N, Sailer CA, Blidl W, Biniossek M, Dehn D, Deller T, Eble S, Abbass K, Wangler T, Knaus HG, Fakler B (2006) The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kv beta 1. *Neuron* 49:697-706.
- Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. In: *Annual Review of Cell and Developmental Biology*, vol. 23, pp 613-643 Palo Alto: Annual Reviews.
- Shiang R, Ryan SG, Zhu YZ, Hahn AF, Oconnell P, Wasmuth JJ (1993) Mutations in the Alpha-1 Subunit of the Inhibitory Glycine Receptor Cause the Dominant Neurologic Disorder, Hyperekplexia *Nature Genetics* 5:351-358.
- Shinnar S, Bello JA, Chan S, Hesdorffer DC, Lewis DV, Macfall J, Pellock JM, Nordli DR, Frank LM, Moshe SL, Gomes W, Shinnar RC, Sun S (2012) MRI abnormalities following febrile status epilepticus in children: The FEBSTAT study. *Neurology* 79:871-877.
- Shinnar S, Pellock JM (2002) Update on the epidemiology and prognosis of pediatric epilepsy. *Journal of Child Neurology* 17:S4-S17.

- Shorvon SD (2011) The etiologic classification of epilepsy. *Epilepsia* 52:1052-1057.
- Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M (1998) A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns. *Nature Genetics* 18:25-29.
- Solimena M, Folli F, Aparisi R, Pozza G, Decamilli P (1990) Autoantibodies to Gabaergic Neurons and Pancreatic Beta-Cells in Stiff-Man Syndrome *New England Journal of Medicine* 322:1555-1560.
- Solimena M, Folli F, Denisdonini S, Comi GC, Pozza G, Decamilli P, Vicari AM (1988) Autoantibodies to Glutamic-Acid Decarboxylase in a Patient with Stiff-Man Syndrome, Epilepsy and Type-I Diabetes-Mellitus. *New England Journal of Medicine* 318:1012-1020.
- Specchio N, Fusco L, Claps D, Vigeveno F (2010) Epileptic encephalopathy in children possibly related to immune-mediated pathogenesis. *Brain & Development* 32:51-56.
- Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH (2006) Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *New England Journal of Medicine* 354:1370-1377.
- Striano P, Belcastro V, Striano S (2011) Tonic seizure: a diagnostic clue of anti-LGI1 encephalitis? *Neurology* 77:2140-2140.
- Su YH, Izumi T, Kitsu M, Fukuyama Y (1993) SEIZURE THRESHOLD IN JUVENILE MYOCLONIC EPILEPSY WITH GRAVES-DISEASE. *Epilepsia* 34:488-492.
- Suleiman J, Brenner T, Gill D, Brilot F, Antony J, Vincent A, Lang B, Dale RC (2011a) VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology* 76:1252-1255.
- Suleiman J, Brenner T, Gill D, Troedson C, Sinclair AJ, Brilot F, Vincent A, Lang B, Dale RC (2011b) Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies. *Developmental Medicine and Child Neurology* 53:1058-1060.
- Suleiman J, Brilot F, Lang B, Vincent A, Dale RC (2013a) Autoimmune epilepsy in children: Case series and proposed guidelines for identification. *Epilepsia*.
- Suleiman J, Wright S, Gill D, Brilot F, Waters P, Peacock K, Procopis P, Nibber A, Vincent A, Dale RC, Lang B (2013b) Autoantibodies to neuronal antigens in children with new-onset seizures classified according to the revised ILAE organization of seizures and epilepsies. *Epilepsia* n/a-n/a.
- Tan KM, Lennon VA, Klein CJ, Boeve BF, Pittock SJ (2008) Clinical spectrum of voltage-gated potassium channel autoimmunity. *Neurology* 70:1883-1890.
- Thieben MJ, Lennon VA, Boeve BF, Aksamit AJ, Keegan M, Vernino S (2004) Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. *Neurology* 62:1177-1182.
- Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T, Honig LS, Benseler SM, Kawachi I, Martinez-Hernandez E, Aguilar E, Gresa-Arribas N, Ryan-Florence N, Torrents A, Saiz A, Rosenfeld MR, Balice-Gordon R, Graus F, Dalmau J (2013) Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurology* 12:157-165.
- Turner MR, Irani SR, Leite MI, Nithi K, Vincent A, Ansorge O (2011) Progressive encephalomyelitis with rigidity and myoclonus Glycine and NMDA receptor antibodies. *Neurology* 77:439-443.
- Urbach H, Soeder BM, Jeub M, Klockgether T, Meyer B, Bien CG (2006) Serial MRI of limbic encephalitis. *Neuroradiology* 48:380-386.
- van Baalen A, Hausler M, Boor R, Rohr A, Sperner J, Kurlmann G, Panzer A, Stephani U, Kluger G (2010) Febrile infection-related epilepsy syndrome (FIREs): A nonencephalitic encephalopathy in childhood. *Epilepsia* 51:1323-1328.
- van Baalen A, Hausler M, Plecko-Startinig B, Strautmanis J, Vlaho S, Gebhardt B, Rohr A, Abicht A, Kluger G, Stephani U, Probst C, Vincent A, Bien CG (2012) Febrile Infection-Related Epilepsy



- Syndrome without Detectable Autoantibodies and Response to Immunotherapy: A Case Series and Discussion of Epileptogenesis in FIRES. *Neuropediatrics* 43:209-216.
- van Baalen A, Stephani U, Kluger G, Hausler M, Dulac O (2009) FIRES: febrile infection responsive epileptic (FIRE) encephalopathies of school age. *Brain & Development* 31:92-93.
- Vezzani A, French J, Bartfai T, Baram TZ (2011) The role of inflammation in epilepsy. *Nat Rev Neurol* 7:31-40.
- Vincent A (2008) Stiff, twitchy or wobbly-are GAD antibodies pathogenic? *Brain* 131:2536-2537.
- Vincent A (2009) Antibodies to Contactin-Associated Protein 2(CASPR2) in Thymoma and Morvan's Syndromet. *Annals of Neurology* 66:S3-S3.
- Vincent A, Bien CG, Irani SR, Waters P (2011a) Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurology* 10:759-772.
- Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J, Vincent A, Buckley C, Schott JM, Baker I, Dewar B-K, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J (2004) Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 127:701-712.
- Vincent A, Crino PB (2011) Systemic and neurologic autoimmune disorders associated with seizures or epilepsy. *Epilepsia* 52:12-17.
- Vincent A, Irani SR (2010) Caspr2 Antibodies in Patients with Thymomas. *Journal of Thoracic Oncology* 5:S277-S280.
- Vincent A, Irani SR, Lang B (2010) The growing recognition of immunotherapy-responsive seizure disorders with autoantibodies to specific neuronal proteins. *Current Opinion in Neurology* 23:144-150.
- Vincent A, Irani SR, Lang B (2011b) Potentially pathogenic autoantibodies associated with epilepsy and encephalitis in children and adults. *Epilepsia* 52:8-11.
- Vincent A, Lang B, Kleopa KA (2006) Autoimmune channelopathies and related neurological disorders. *Neuron* 52:123-138.
- Vitaliani R, Mason W, Ances B, Zwerdling T, Jiang ZL, Dalmau J (2005) Paraneoplastic encephalitis, psychiatric symptoms, and hypoventilation in ovarian teratoma. *Annals of Neurology* 58:594-604.
- Vulliemoz S, Vanini G, Truffert A, Chizzolini C, Seeck M (2007) Epilepsy and cerebellar ataxia associated with anti-glutamic acid decarboxylase antibodies. *Journal of Neurology Neurosurgery and Psychiatry* 78:187-189.
- Waites KB, Talkington DF (2004) *Mycoplasma pneumoniae* and its role as a human pathogen. *Clinical Microbiology Reviews* 17:697-+.
- Watson R, Jiang Y, Bermudez I, Houlihan L, Clover L, McKnight K, Cross JH, Hart IK, Roubertie A, Valmier J, Hart Y, Palace J, Beeson D, Vincent A, Lang B (2004) Absence of antibodies to glutamate receptor type 3 (GluR3) in Rasmussen encephalitis. *Neurology* 63:43-50.
- Weber YG, Lerche H (2008) Genetic mechanisms in idiopathic epilepsies. *Developmental Medicine and Child Neurology* 50:648-654.
- WHO (2010) International Statistical Classification of Diseases and Related Health Problems 10th Revision, Chapter VI Diseases of the nervous system.
- Wilder-Smith EPV, Lim ECH, Teoh HL, Sharma VK, Tan JJH, Chan BPL, Ong BKC (2005) The NORSE (new-onset refractory status epilepticus) syndrome: Defining a disease entity. *Annals Academy of Medicine Singapore* 34:417-420.
- Wong-Kisiel LC, McKeon A, Wirrell EC (2012) Autoimmune Encephalopathies and Epilepsies in Children and Teenagers. *Canadian Journal of Neurological Sciences* 39:134-144.
- Wong SH, Saunders MD, Larner AJ, Das K, Hart IK (2010) An effective immunotherapy regimen for VGKC antibody-positive limbic encephalitis. *Journal of Neurology Neurosurgery and Psychiatry* 81:1167-1169.

- Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. *Nature Reviews Drug Discovery* 8:982-1001.
- Yellen G (2002) The voltage-gated potassium channels and their relatives. *Nature* 419:35-42.
- Yu N, Liu H, Di Q (2013) Modulation of Immunity and the Inflammatory Response: A New Target for Treating Drug-resistant Epilepsy. *Current Neuropharmacology* 11:114-127.
- Zuliani L, Andriago C, Leite MI, Casano A, Waters P, Woodhall M, Dalla Mora E, Morra M, Giometto B, Vincent A (2011) Immunotherapy-Responsive Status Epilepticus with Anti-Glycine Receptor Antibody. *European Journal of Neurology* 18:50-50.
- Zuliani L, Graus F, Giometto B, Bien C, Vincent A (2012) Central nervous system neuronal surface antibody associated syndromes: review and guidelines for recognition. *Journal of Neurology Neurosurgery and Psychiatry* 83:638-645.