Autoimmune Encephalitis: The clinical value of antibodies directed to extracellular antigens

Auto-immuun encefalitis: de klinische waarde van antilichamen gericht tegen extracellulaire antigenen

door

Agnes van Sonderen

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Table of contents

CHAPTER 1

General introduction

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History and classification of antibodies in neurology

The discovery of antibodies in tumor patients was a major step in the field of antibody-associated neurological syndromes. The Dutch Professor B. Brouwer (1881-1949) reported cerebellar degeneration in a patient with a pelvic tumor in 1919. He was the first to link neurological diseases to remote tumors, considering a toxic effect.[1] The association of limbic encephalitis and tumors was reported half a century later. One of the first patients was a 60-years old bus driver, referred to the hospital in 1960 with complaints of weakness and weight loss. In the following weeks, he had seizures, confusion and complete loss of memory for the previous eight weeks. At that time, extensive investigation consisting of blood examination, cerebrospinal fluid (CSF) analysis and electroencephalogram (EEG) did not show a cause for his illness. In the following two years, disease worsened and he suddenly died in 1961. Post-mortem examination revealed a bronchial carcinoma. Death was attributed to an "unidentified cerebral illness", but this case, together with a few similar cases, raised the question whether there might be an association between inflammatory brain disease and tumors:

"THERE are several kinds of neurological disorder which may develop in patients with carcinoma, even though no manifest spread of tumour cells to the nervous system has occurred. (…) Both the degenerative and the inflammatory changes have generally been considered to occur only at levels caudal to the basal ganglia. In recent years, however, evidence has been accumulating that the cerebral hemispheres may be affected rather than the hind-brain.(…) The damage moreover has been severe at times and there has then been a noticeable tendency for the patients to develop memory disturbances or to be demented. The first question to arise therefore is whether the assertion of a connexion between carcinoma and "limbic encephalitis" is now justified."

Corsellis. Brain, 1968[2]

Although the opportunities for ancillary testing have greatly expanded, our findings are not much different than fifty years ago: severely affected patients, with minimal or aspecific abnormalities at extensive examinations. Fortunately, we are now able to make a diagnosis in the majority of these patients, based on antibody-detection. Prognosis and treatment options have improved, but are mainly dependent on the antibodies' target: intracellular vs extracellular antigens.

The 'classical paraneoplastic antibodies' are directed to intracellular proteins, such as Hu, Ri and Yo. Due to its intracellular target, these antibodies are probably not directly pathogenic. Antibodies are thought to occur as an epiphenomenon of a hypothesized T-cell mediated inflammation. This inflammation results in mostly irreversible neuronal damage, and therefore the effect of immunotherapy is limited.[3,4] There is a strong antibody-dependent tumor association. For example, 85% of the anti-

Hu patients have a small cell lung cancer, while patients with anti-DNER should be analyzed for Hodgkin lymphoma. Because of the remarkable tumor association, these diseases as often referred to as 'paraneoplastic antibodies', but the term 'onconeural antibodies' is preferred.

In the year 2007, the discovery of N-methyl-d-aspartate receptor (NMDAR) antibodies was a major breakthrough recognizing cell surface proteins as antigens in encephalitis.[5] Several other cell surface or synaptic antigens and their clinical syndrome have been reported more recently, including the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) in 2009 and leucine-rich glioma-inactivated protein1 (LGI1) and contactin-associated protein-like 2 (Caspr2) in 2010.[6-8] In contrast to the classical syndromes, these antibodies are thought to be directly pathogenic and patients tend to have a more favorable response to immunotherapy. Only a minority of these patients have an associated tumor, although the incidence of cancer differs per antigen: 30-40% of the anti-NMDAR encephalitis patients have a tumor, while tumors occur in only 11% of the patients with antibodies to LGI1.[6,8,9]

This chapter gives an overview of the syndromes associated with antibodies directed to extracellular antigens. Antibodies related to the presynaptic voltage-gated potassium channel (VGKC) complex are discussed first, including LGI1 and Caspr2. Subsequently, the postsynaptic glutamate receptors AMPA and NMDA are discussed. Extensive descriptions of antibodies to glycine, dipeptyl-peptidase-like protein-6 (DPP6) and γ-aminobutyric acid (GABA) receptor type B and type A are beyond the scope of this thesis, but a short summary is added to this chapter.

The presynaptic voltage-gated potassium channel (VGKC) complex

Introduction

Antibodies to the VGKC were initially detected in patients with acquired neuromyotonia, a peripheral nerve disorder characterized by muscle cramps, impaired relaxation and stiffness.[10] A pathogenic role of VGKC antibodies was subsequently suspected in Morvan's syndrome, showing neuromyotonia accompanied by autonomic and cognitive symptoms and insomnia,[11] and in patients with limbic encephalitis.[12] Antibodies were eventually thought to be directed to subunits of the VGKC receptor.[13] However, the exact role of VGKC-antibodies remained controversial as no laboratory succeeded in showing staining with serum in VGKC-transfected cells. In the year 2010, this reconsideration led two laboratories to identify simultaneously that these antibodies are not directed to the subunits of the VGKC itself, but to VGKC-associated proteins: LGI1 and Caspr2.[6,8] The VGKC-test is a commercially available 125-I-α-dendrotoxin radioimmunoassay (RIA). Values and cut off values vary among laboratories; usually titer > 100 pM or > 400 pM are interpreted as positive. The test is only applicable on serum and is not able to discriminate sera with antibodies to LGI1, Caspr2 or neither of them. This relevant

distinction can be made with immunocytochemistry or immunohistochemistry, using serum or CSF.

Leucine-rich glioma-inactivated 1 (LGI1)

The LGI1 protein is secreted into the synaptic cleft, where it binds a disintegrin and metalloprotease domain-containing protein 23 (ADAM23) to postsynaptic ADAM22, thereby influencing synaptic transmission to the AMPAR.[3,14] This transsynaptic fine tuning is thought to have an anti-epileptic effect.[14] Patients with antibodies to LGI1 show the typical features of limbic encephalitis: seizures, memory deficit, confusion and behavioral problems. Typical for anti-LGI1 encephalitis are faciobrachial dystonic seizures (FBDS). These are brief involuntary unilateral movements involving the arm, usually ipsilateral face and less commonly the trunk or a leg. FBDS occur very frequent, up to 100 times per day, but are seen in only half of the patients.[15,16] No trials analyzed the effect of immunotherapy in anti-LGI1 encephalitis, but treatment response is series is mostly favorable.[17,18] Until recently, extensive analysis of long-term outcome was lacking.

We analyzed a cohort of 38 Dutch anti-LGI1 patients to clarify the clinical syndrome in more detail, giving clues for clinical recognition of this relatively 'new' disease. Long-term outcome was analyzed, including neuropsychological assessment and relapse rates. **(Chapter 2)**

In the clinical setting, we noticed a common HLA-DRB1*07 (DR7) allele in our anti-LGI1 patients. For systematic analysis, we performed HLA-phenotyping in a larger group of anti-LGI1 patients. The aim was to analyze the association between HLA-type and predisposition for anti-LGI1 encephalitis in non-tumor patients. **(Chapter 3)**

Contactin-associated protein-like 2 (Caspr2)

Caspr2 is a membrane protein in myelinated axons in both the central and peripheral nervous system, essential to stabilize the VGKC's at the juxtaparanodes.[6,19] Disruption of the Caspr2 protein is thought to diminish repolarization, causing hyperexcitability. Mutation in the Caspr2 coding gene, CNTNAP2, causes childhood onset refractory epilepsy with mental retardation.[3] Only a few dozen patients with Caspr2 antibodies had been published before we started our analysis. The majority of these patients were male, and most patients presented with limbic encephalitis, neuromyotonia or a combination of central and peripheral nerve system symptoms known as Morvan's syndrome. Again, treatment trials are lacking, but patient series report a good response to immunotherapy.[6,19]

To gain more insight in the disease, we analyzed the largest cohort of 38 anti-Caspr2 patients. We studied the overlap in earlier described syndromes, defined the core symptoms and analyzed treatment responses. IgG subclasses were tested in serum. **(Chapter 4)**

VGKC-positivity in the absence of antibodies to LGI1 and Caspr2

A significant part of the VGKC-positive patients do not have LGI1 or Caspr2 antibodies. Unfortunately, many studies (including over 800 patients) do not distinguish this third group in there analysis, complicating the extraction of group-specific data.[20-25] VGKC-positive patients lacking antibodies to LGI1 and Caspr2 can present with the typical clinical syndromes of limbic encephalitis or neuromyotonia.[10,26,27] But in recent years, the clinical spectrum has expanded: pain syndromes, psychogenic non-epileptic seizures, REM sleep behavior disorder, multiple system atrophy, peripheral neuropathy, vasculitis, seizures and many other clinical syndromes were reported.[21,23,28-30] In addition, VGKC-positivity was reported in patients with pathology-proven Creutzfeldt Jakob Disease.[31]

The clinical heterogeneity raised the question whether VGKC-positivity in the absence of LGI1 and Caspr2 antibodies is clinically relevant in all. Several studies addressed this issue by comparing patients with high and low VGKC-titers, and concluded that higher titers are associated with autoimmune disease. However, patients with LGI1 and Caspr2 antibodies (known to have high titers) were not excluded. Others conclude that the clinical relevance of VGKC-positivity is supported by the favorable response to immunotherapy. For example, symptoms improved after immunotherapy in 8/10 patients with pain syndromes and in 3/4 patients with seizures.[24,32] These results seem promising. However, conclusions regarding clinical relevance of VGKC-positivity require a comparison with VGKC-negative patients. Therefore, we compared 25 VGKC-positive patients without LGI1 and Caspr2 antibodies to 50 VGKC-negative patients, matched by age, gender and clinical syndrome. We compared criteria for autoimmune inflammation, treatment responses and VGKC titers between the two groups. We aimed to analyze whether VGKC-positivity in the absence of antibodies to LGI1 and Caspr2 is clinically relevant. **(Chapter 5)**

A review of the etiology, pathogenesis and clinical syndromes caused by antibodies to LGI1 and Caspr2, as well as the complex issue of VGKC-patients lacking both antibodies, is given in **Chapter 6**.

The postsynaptic glutamate receptors

Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)

In 2009, 43 patients with limbic encephalitis of unknown origin were investigated in order to identify the antigen. Among these, antibodies of ten patients showed a similar pattern of reactivity to neuropil of rat brain and cerebellum. Further tests showed that the AMPAR was the target antigen in these patients.[7] The AMPAR is an ionotropic glutamate receptor concentrated at synapses, mediating most of the fast excitatory neurotransmission in the brain.[33] Antibody reaction leads to a decrease in the number of receptors at synapses and a decrease of receptors along dendrites due to increased

internalization of AMPARs. Removal of antibodies from neuronal cultures has shown to restore receptor number and localization of AMPAR clusters.[7]

Two series described just over a dozen patients altogether.[7,34] Most patients were women > 50 years of age. Patient usually presented with limbic encephalitis with memory disorder, confusion and often psychiatric symptoms. Seizures were less common compared to other antibodies.

We further characterized this clinical syndrome in 22 newly identified patients with anti-AMPAR-encephalitis. Co-occurrence of other antibodies, response to immunotherapy and clinical outcome were analyzed. **(Chapter 7)**

N-methyl-d-aspartate receptor (NMDAR)

In 2005, four young women were described with acute psychiatric symptoms, memory deficit, seizures, decreased level of consciousness and central hypoventilation. All four had ovarian teratoma. CSF showed a common pattern of reactivity to the cytoplasmic membrane of hippocampal neurons.[35] In 2007, this had led to the identification of the NMDAR as the target antigen.[5]

Binding of antibodies to the NMDAR results in prolongation of the opening time of the receptor.[36] This hyperfunction might induce excessive calcium influx, resulting in damage to the receptor, although the exact mechanism is yet undetermined. In neuronal cultures, patients' antibodies are shown to induce capping and internalization of the NMDAR, resulting in a (reversible) decrease of the amount of NMDAR clusters.[37-39] Injection of patient CSF in mice was shown to decrease hippocampal NMDARs, causing reversible memory and behavioral deficits in the animal.[40] Unprovoked NMDAR-encephalitis in animals occurs as well: the popular polar bear Knut drowned during a seizure in the Berlin Zoo in 2011. Post-mortem, he appeared to have high concentrates of NMDAR-antibodies in his CSF.[41]

In humans, NMDAR-antibodies are the most common cause of antibody-mediated encephalitis. The largest cohort describes 577 patients, of which 80% are female. The vast majority of the patients is between 18 and 45 years. In this observational study, 38% of the patients had a tumor, of which 94% were ovarian teratoma.

Half of the patients have prodromal symptoms suggesting a nonspecific viral infection.[9] In the following days to weeks patients, develop psychiatric symptoms, short term memory deficit, confusion, insomnia and language deterioration.[42,43] Abnormal movements, such as orofacial dyskinesias and chorea, are common. Subsequently, level of consciousness decreases and autonomic instability and hypoventilation may occur, requiring admission to the intensive care unit in the majority. Recovery from anti-NMDAR-encephalitis occurs in the reverse order of symptom presentation.[9] Trials concerning treatment of anti-NMDAR encephalitis have not been performed, but large series show a convincing effect. Treatment usually consists of a combination of methylprednisolone and intravenous immunoglobulins, and tumor removal if

applicable. Over half of the patients respond within four weeks.[9] If first line therapy fails, second line therapy with rituximab and cyclophosphamide can be initiated. However, in extreme cases, response is lacking after adequate first and second line treatment. We report a severe case of anti-NMDAR encephalitis, and discuss treatment considerations after first and second line failure. This is the first report of intrathecal administration of rituximab. (**Chapter 8**)

EEG usually shows diffuse background slowing. One fourth of the patients have electrographic seizures.[9] A unique EEG pattern has characterized in anti-NMDAR encephalitis, consisting of rhythmic delta activity with superimposed bursts of beta activity.[44,45] This pattern of 'extreme delta brushes' was named after the delta brush EEG pattern known in premature infants. Besides the presence of extreme delta brushes in the minority of the patients, data regarding EEG in anti-NMDAR encephalitis was limited. We analyzed first EEG and follow up registrations in 53 adult and pediatric patients. Initial EEG and follow up registrations were re-evaluated. Besides describing the most relevant EEG patterns, the study focuses on the predictive value of first EEG recordings. (**Chapter 9**)

Metabotropic glutamate receptors: mGluR5 and mGluR1

The NMDA receptor and AMPA receptor are ionotropic glutamate receptors. In contrast, there are several metabotropic subtypes of glutamate receptors (mGluR). These receptors indirectly activate ion channels. A few patients with antibodies directed to the mGluR5 or mGluR1 subtype have been reported.[3,46]

Antibodies to mGluR5 cause the 'Ophelia syndrome', named after the character in Shakespeare's Hamlet. The syndrome was first described in 1982 by Dr Ian Carr. He wrote a moving personal paper about the subacute loss of memory and psychosis in his fifteen years old daughter Jane, who subsequently appeared to have Hodgkin lymphoma.[47] More recently three patients with a comparable clinical picture in Hodgkin lymphoma were reported. In these patients mGluR5 was detected as the target antigen of the antibodies.[46,48,49] All patients had a favorable outcome, similar to Carr's daughter.

Antibodies to mGluR1 are described in five patients with subacute cerebellar ataxia.[49 46,50,51] The pathogenic role of mGluR1 antibodies has been demonstrated by the induction of cerebellar symptoms in mice after passive transfer of patient's antibodies.[49] Neurological outcome in the five reported patients is variable, despite immunotherapy.

Beyond the scope of this thesis

The major progressions made in the field of antibody-mediated neurologic disease has led to the discovery of a number of other extracellular antigens. A short description of the four most prominent follows here, but an extensive description is beyond the scope of this thesis.

Antibodies to the glycine receptor (GlyR) are detected in patients with progressive encephalomyelitis with rigidity and myoclonus (PERM) or stiff person syndrome (SPS). GlyRs are chloride channels on the cell surface membrane, facilitating inhibitory neurotransmission in the brain and spinal cord. Receptor dysfunction leads to abnormal discharges of motor neurons and widespread muscular rigidity.[52,53] Case reports describe beneficial effect of immunotherapy, but patients tend to relapse.

Antibodies to the dipeptyl-peptidase-like protein-6 (DPP6, or DPPX) were identified in four patients with rapidly progressive encephalopathy in the year 2012. Interestingly, three of these patients had severe prodromal diarrhea.[54] In 2014, DPP6 antibodies were detected in three patients with PERM.[55] DPP6 is a cell surface subunit of the Kv4.2 potassium channel, most prominent in hippocampal neurons. DPP6 is present in the myenteric plexus as well, explaining diarrhea in a part of the patients.[3,54]

The γ-aminobutyric acid-B (GABAB) receptor was identified as target antigen in limbic encephalitis in 2010. GABAB receptors have an inhibitory function both presynaptic and postsynaptic. Seizures, often refractory, are prominent in anti-GABAB patients. The majority of the patients have memory deficit and confusion as well, meeting the criteria for limbic encephalitis.[56-58] Over half of the patients have a tumor, mainly small cell lung cancer (SCLC).[56] The majority of the patients respond well to immunotherapy.

In 2014, two patients with encephalitis and refractory seizures showed an immunohistochemistry pattern similar to GABAB receptor antibodies, but specific testing for GABAB was negative. In these two patients, and in four others, antibodies to the GABAA receptor were detected.[59] Patients had a rapidly progressive encephalopathy resulting in refractory seizure, mostly status epilepticus.

Hypothesis

The chapters refer to the studies answering these hypotheses:

- Anti-LGI1 encephalitis is greatly underdiagnosed. More insight in the clinical features will be the key to improve recognition. (**Chapter 2**)
- Long-term outcome in anti-LGI1 encephalitis is mostly favorable, but residual cognitive deficits are common. (**Chapter 2**)
- Anti-LGI1 encephalitis is associated with a HLA-subtype, supporting the autoimmune hypothesis. (**Chapter 3**)
- Anti-LGI1 encephalitis and anti-Caspr2 encephalitis can mimic dementia. (**Chapters 2** and **4**)
- Anti-Caspr2 encephalitis can present with various syndromes, but there is substantial overlap in the main symptoms. (**Chapter 4**)
- In many patients, VGKC-positivity in the absence of antibodies to LGI1 or Caspr2 is not clinically relevant. (**Chapter 5**)
- AMPAR-antibodies are associated with a treatment-responsive limbic encephalitis, often with psychiatric symptoms. (**Chapter 7**)
- Tumor incidence is high in anti-AMPAR-encephalitis. (**Chapter 7**)
- Response to therapy and clinical recovery in anti-NMDAR encephalitis can be delayed. (**Chapter 8**)
- EEG is predictive for clinical outcome in anti-NMDAR encephalitis. (**Chapter 9**)

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PART I:

The presynaptic VGKC-complex

CHAPTER 2

Anti-LGI1 encephalitis: clinical syndrome and long-term follow-up

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Abstract

Objectives

This nationwide study gives a detailed description of the clinical features and long-term outcome of anti-LGI1 encephalitis.

Methods

We collected patients prospectively from October 2013, and retrospectively from samples sent to our laboratory from January 2007. LGI1-antibodies were confirmed with both cell-based assay and immunohistochemistry. Clinical information was obtained in interviews with patients and their relatives and from medical records. Initial MRI and follow-up MRI were revised blindly. Neuropsychological assessment was performed in those patients with follow-up over two years.

Results

Annual incidence in the Netherlands was 0.83/million. 34/38 patients had a limbic encephalitis. Subtle focal seizures (66%, autonomic and/or dyscognitive) and faciobrachial dystonic seizures (FBDS, 47%) mostly occurred before onset of memory disturbance. Later in disease course, 63% had tonic-clonic seizures. Initial MRI showed hippocampal T2 hyperintensity in 74% of the patients. These lesions evolved regularly into mesotemporal sclerosis (44%). Substantial response to immunotherapy was seen in 80%, with early response of seizures and slow recovery of cognition. At follow-up ≥2 years, most surviving patients reported mild residual cognitive deficit with spatial disorientation. 86% had persistent amnesia for the disease period. Relapses were common (35%) and presented up to eight years after initial disease. 2-years case fatality rate was 19%.

Conclusions

Anti-LGI1 encephalitis is a homogenous clinical syndrome, showing early FBDS and other focal seizures with subtle clinical manifestations, followed by memory disturbances. Better recognition will lead to earlier diagnosis, essential for prompt start of treatment. Long-term outcome of surviving patients is mostly favorable, but relapses are common.

Introduction

Antibodies directed to leucine-rich glioma-inactivated 1 (LGI1) were discovered in 2010.[1,2] Before, patients were thought to have antibodies against voltage-gated potassium channels (VGKC), to which the LGI1-protein is functionally related. Most anti-LGI1 patients present with limbic encephalitis (LE). LE is clinically characterized by a subacute disturbance of memory and behavior, often accompanied by seizures. Patients tend to improve on immune therapy, but long-term outcome is characterized poorly.

LGI1 is mainly expressed in the hippocampus and the temporal cortex, where it is secreted into the synaptic space. It is part of an inhibitory pathway linking the presynaptic VGKC and the postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid-receptor (AMPAR).[3,4] Genetic disruption of the LGI1-protein causes autosomal dominant lateral temporal epilepsy.[5,6] Seizures are common in patients with LGI1-antibody-mediated disease as well. Faciobrachial dystonic seizures (FBDS) are very specific for anti-LGI1 encephalitis, although only present in a minority of the patients.[7] FBDS are involuntary contractions of 1-2 seconds, affecting the unilateral arm (or leg) and face, occurring up to 100 times a day, but often unrecognized by patients and physicians.

Approximately 250 anti-LGI1-patients have been reported, mostly described as "VGKC-encephalitis". Due to better recognition, incidence is expected to increase dramatically, but data regarding incidence are scarce. This nationwide study provides the opportunity to report incidence rates. We describe the disease course in detail, and provide clues to improve clinical recognition and avoid laboratory pitfalls. We report long-term outcome, including neuropsychological assessment and relapse rates.

Methods

Patients accrual and laboratory testing

Samples had been sent for antibody testing to the laboratory of Medical Immunology of the Erasmus University Medical Center, Rotterdam. This national referral center is the only laboratory performing LGI1-antibody analysis in the Netherlands. Patients with confirmed LGI1-antibodies were included irrespective of age or clinical syndrome. Patients diagnosed between 2007 and October 2013 were included retrospectively and invited for neuropsychological assessment to analyze long-term cognitive outcome. Patients diagnosed between October 2013 and October 2015 were assessed prospectively.

LGI1-antibodies were detected with both cell-based assay and immunohistochemistry in serum (and CSF if available) as previously reported.[9]

Incidence rate was based on the number of patients diagnosed in the Netherlands in the last twelve months (October 2014-September 2015), and compared to the three-year period before this study started (Oct 2010-Sept 2013).

Clinical information

Clinical information was obtained in an interview with patient and relatives (if possible) during visit to our clinic (n=21) or by telephone (n=17), and from medical records and telephone interviews with the treating physicians.

Seizures were classified by an experienced epileptologist (RT), based on interviews, files and EEG reports. FBDS were defined as frequent seizures (> 8/day) with a dystonic posture of the arm, often accompanied by facial contraction, lasting less than 30 seconds.[10] Current sleep complaints were assessed with Epworth Sleepiness Scale (ESS) for daytime sleepiness and Pittsburgh Sleep Quality Index (PSQI) for sleep quality and disturbances. Patients were considered responsive to treatment if they showed substantial clinical improvement within four weeks after the start of treatment, as judged by the treating physicians.

Brain MRIs performed during and after disease were re-evaluated by an independent experienced neuroradiologist (ES), mixed with control MRIs from antibody-negative epilepsy patients for blinding. Visual assessment of hippocampal volume and FLAIR or T2-weighted signal intensity changes within the hippocampus were performed. The evaluation was performed on coronal plane images at the level of the hippocampus (or axial plane when coronal images were absent).

Long-term cognitive outcome was analyzed in patients with disease onset over two years ago. Neuropsychological assessment was performed using Cambridge Neuropsychological Test Automated Battery (CANTAB Research Suite 6.0, Cambridge Cognition Ltd., Cambridge, UK). We expected most relevant residual deficits in domains of memory, spatial orientation and executive functioning. Therefore, our primary outcomes were the Spatial Recognition Memory (SRM) and Intra-Extra Dimensional set shift (IED, total errors) test results. Seven tests of different cognitive domains were added for wider exploration of cognitive outcome (Suppl. Table e-1).

Standard protocol approvals, registrations and patient consents

The study was approved by the Institutional Review Board of the Erasmus University Medical Center, Rotterdam. Informed consent was obtained in all patients.

Statistical analysis

Incidence rate was calculated with 95% confidence intervals (CI) based on a Poisson distribution, using available population data (http://statline.cbs.nl/statweb/). Categorical data were analyzed with Fisher-Freeman-exact test, numerical data with Mann Whitney-U test, and correlation by Spearman's rho, with p-values <0.05 considered significant. CANTAB results were expressed as z-scores, based on normative data (age and gender specific) obtained by the company and analyzed with one-sample T-tests (Test Value=0). Primary outcome measures (SRM and IED) were considered significant if $p<0.025$ (Bonferroni). Secondary outcome measures were exploratory,

without correction for multiple testing. These p-values should be considered carefully. SPSS Statistics 21 (IBM Corp., Armonk, NY) was used for analysis.

Results

Thirty-nine anti-LGI1 encephalitis patients were identified, of whom 38 patients were available for inclusion (19 prospectively; Table 1). Twenty-five patients were male (66%). Median age at onset of disease was 64 years. Median follow-up was 27 months. Long-term outcome was analyzed in 21 patients with follow-up ≥2 years.

Incidence

Fourteen patients were clinically diagnosed with anti-LGI1 encephalitis in the last year, resulting in an annual incidence of 0.83/million (95%-CI 0.45-1.40), an increase compared to only 11 patients diagnosed in the three-year period earlier (annual incidence 0.22/million; 95%-CI 0.11-0.39; p=0.002).

Clinical phenotype

Thirty-four patients had limbic encephalitis, three patients had Morvan's syndrome (limbic encephalitis and peripheral nerve hyperexcitability and insomnia/dysautonomia) and one patient had only seizures. Most common initial symptoms were seizures (53%) or cognitive disorder (42%; Figure 1A). Typical disease course is shown in Figure 1B. Median time from onset to nadir of disease was 22 weeks. At maximum disease severity, 76% had mRS ≥3. Almost all patients evolved disturbance of memory (97%) or behavior (90%). Behavioral changes included apathy (53%), disinhibition (excessive eating, missing social cues, 40%), egocentrism (38%), or compulsive behavior (clean up, hoarding, 29%). Over half of the patients had spatial disorientation, often reported as getting lost walking to the supermarket or at the ward.

Table 1. Patient characteristics (n = 38)

Male gender	25/38 (66%)
Age at onset in years, median (IQR, range)	64 (60 – 69, 31 to 84)
Time to maximum disease severity in weeks (IQR, range)	22 (8 – 32, 2 to 150)
Clinical syndrome - Limbic encephalitis - Morvan's syndrome # - Epilepsy	 34 (90%) 3 (8%) 1 (3%)
Seizures	34 (90%)
Memory deficit	37 (97%)
Disorder of behavior	34 (90%)
Spatial disorientation	17/33 (52%)
Insomnia	20/31 (65%)
Weight loss	9/33 (27%)
Autonomic dysfunction	15/32 (47%)
Pain	3/34 (9%)
Peripheral nervous system symptoms	5/32 (16%)
Hyponatremia	24/37 (65%)
CSF - Cell count > 5 cells/uL - Protein > 0.58 g/L	 - 5/32 (16%) (max 88 cells /μL) - 5/32 (16%)
EEG - Focal slowing - Epileptic	 - 9/36 (25%) - 11/36 (31%)
MRI, at presentation - Unilateral hippocampal lesion - Bilateral hippocampal lesion - Normal	 - 21/35 (60%) - 5/35 (14%) - 9/35 (26%)
MRI, at follow-up (with initial hippocampal lesions) - Mesotemporal sclerosis - Hippocampal T2 hyperintensity - Normal	 - 7/17 (41%) - 6/17 (35%) - 4/17 (24%)
- Tumor	- 4/36 (11%)
- VGKC RIA median (IQR, range)	- 720 (457 – 971, 245 to 1314)
- Cell-based assay LGI1. Serum; CSF†	- 38/38 (100%); 9/17 (53%)
- Immunohistochemistry LGI1. Serum; CSF††	- 38/38 (100%); 15/17 (88%)*

Morvan's syndrome was defined as limbic encephalitis with peripheral nerve hyperexcitability and sleep disorder or dysautonomia; †p= 0.008, McNemar's test comparing serum with CSF. ††p = 0.50, McNemar's test comparing serum with CSF. *Both negative scored samples were negative on cell-based assay as well. IQR = interquartile range, FBDS = faciobrachial dystonic seizures, RIA = radioimmunoassay.

During the course of disease, 33 patients (89%) developed one or more types of seizures (Table 2, case descriptions in Supplement). FBDS (47%) started mostly several weeks before the onset of cognitive symptoms. Median frequency was 40 FBDS per day. Focal seizures with mainly dyscognitive (n=15), autonomic (n=9), motor (n=3) or gelastic (n=2) features or aura (n=1) were present in 25 patients (66%). Focal seizures lasted longer than FBDS and the median frequency was 12 per day. Tonic-clonic seizures

occurred in 63% of the patients, mostly simultaneous with or after onset of cognitive decline. Most patients had only a single or a few tonic-clonic seizures (median 3). This last type of seizures was often the trigger to start or extend ancillary testing.

Other common symptoms were insomnia (65%) and autonomic dysfunction (47%, mainly hyperhidrosis). Sexual dysfunction was not systemically questioned but 5 male patients (21%) reported it spontaneously. One patient had marked chorea. One patient evolved bulbar symptoms of myasthenia gravis, concomitant with the start of limbic encephalitis (AChR antibodies positive, no thymoma).

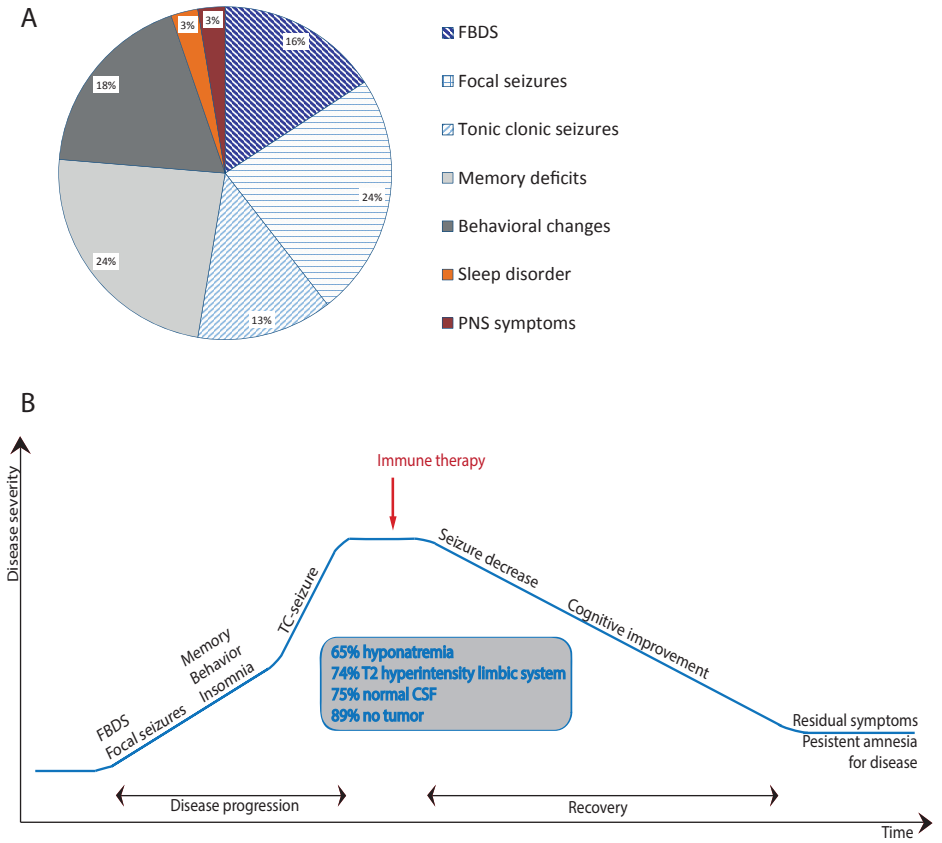

Figure 1. Presenting symptoms and disease course. A. First symptom in 38 patients with anti-LGI1 encephalitis. B. Disease course in anti-LGI1 encephalitis. Timeline: median disease progression 22 weeks, median treatment delay 25 weeks, median start of improvement 2 weeks after treatment, median time of recovery 33 weeks. FBDS = faciobrachial dystonic seizures. TC-seizure = tonic-clonic seizure.

Table 2. Seizure characteristics in 38 patients

	FBDS	Focal seizures (other than FBDS)	Tonic-clonic seizures
Number of patients	18 (47%)	25 (66%)	24 (63%)
Duration of seizure	< 15 seconds	Median: 25 sec (range 1 – 600)	
Seizure frequency	Median: 40/day Range: 10-100/day IQR: 20 – 80	Median: 12/day Range: 1-150/day IQR: 3 – 40	Median: 3 in total Range: 1-100 in total IQR: 1 – 5
Relation to onset of cognitive symptoms	Before (67%) Median 3 weeks before	Before or simultaneous (90%) Median 1.5 weeks before	Simultaneous or after (78%) Median 0.5 weeks after

FBDS = faciobrachial dystonic seizures, IQR = interquartile range

Ancillary testing

In the acute symptomatic phase, hyponatremia was found in 65% of the patients. CSF cell count and protein were unremarkable in 75%. Initial brain MRI was available for revision in 35 patients (Suppl. Figure e-2A). Median time from onset to first MRI was 11 weeks (IQR 5–24, range 0-55 weeks). Hippocampal lesions were seen in 74%, of which 81% were unilateral. 40% had swelling of the hippocampus with increased T2-signal intensity. 14% had a hyperintensity on T2-images with normal hippocampal volume. Seven (20%) patients had loss of hippocampal volume on initial MRI. Six of these seven patients had increased T2-signal intensity, of which four fulfilled criteria for mesotemporal sclerosis (MTS). Median time from onset was 6.5 weeks in patients with swelling and 18.5 weeks in patients with loss of hippocampal volume (p=0.052). In 23%, hyperintensities extended to the amygdala (n=6), insula (n=1) or striatum (n=1).

Follow-up MRI's were available in 19 patients (Suppl. Figure e-2B). Median time from symptom onset was 27 weeks (range 7-149 weeks). 16 patients had hippocampal lesions on first MRI, of which seven patients (44%) had MTS at follow-up (three with MTS at initial MRI). 86% of patients with MTS had multiple seizures daily during maximal disease severity, compared to 50% in patients without MTS (p=0.17). 38% had persistent high signal without loss of volume. Brain MRI had normalized in the other three patients (19%) and three patients with initial normal MRI still had normal MRI at follow-up.

Electroencephalography (EEG) showed epileptic discharges (31%) or focal slowing (25%) in half of the patients. 13 patients had clinical manifestations of seizures during EEG recordings. FBDS had no EEG correlate (n=7), while 16/17 dyscognitive, autonomic, gelastic or motor focal seizures were associated with epileptic discharges.
Tumor analysis showed malignancy in three patients: neuroendocrine pancreas tumor, thymoma with metastasis and abdominal mesothelioma. An additional patient had rectal carcinoma in situ detected two months before onset of neurological disease. A fifth patient had metastatic breast cancer for seven years and was excluded from tumor

analysis.[11] Although unknown if all tumors were related, we calculated tumor incidence as 4/36 (11%).

Median VGKC-RIA result was 720 pM, ranging from 245 to 1314 pM (positive >100pM). All sera tested positive for LGI1-antibodies on both CBA and IHC (Suppl. Figure E-1). CSF was available for testing in 17 patients, of which 88% showed typical LGI1-antibody staining on rat brain. Only 9/17 CSF samples (53%) were positive for LGI1 by CBA.

In all tested patients, no antibodies to Caspr2 (n=38), NMDA-receptor (n=32) or onconeural antibodies (n=29) were found.

Treatment

Initial disease episode was treated with immunotherapy in 32 patients, with a median delay of 25 weeks (IQR 9-46, range 1 week to 2.5 years). 30 patients were treated with corticosteroids (oral n=8, intravenous n=6 or both n=16), of which 18 were additionally treated with intravenous immunoglobulins or plasma exchange. Two patients received only immunoglobulins. First-line treatment was considered effective in 80% of the patients. Median time to start of improvement was two weeks. Improvement started with decrease of seizures in 58% or decrease of seizures simultaneous with cognitive improvement in 42%.

Six patients were not treated with immunotherapy. Three patients, two diagnosed retrospectively, mainly suffered from seizures, with only minor cognitive symptoms, and improved on anti-epileptic drugs. A fourth patient was initially diagnosed with complex partial seizures, but when she developed behavioral problems she was suspected to have psychiatric disease. She remained untreated until diagnosis at relapse. Two patients had severe limbic encephalitis and died untreated without diagnosis.

Long-term follow-up

Follow-up over two years was available in 21 patients. Median follow-up was 42 months. 67% had a favorable outcome (mRS 0-2, Figure 2). Two patients (10%) were moderately affected (mRS=3) due to a relapse. Two patients initially showed partial recovery, but died due to comorbidities aggravated by steroids (spinal cord injuries due to thoracic fracture and diverticular perforation) and three patients died without initial improvement. Two-year case fatality rate was 19%.

In recovering patients, median time between start of improvement and end of recovery was 33 weeks (IQR 18-52, range 13-108). Seizures were reported to recover early. At final follow-up, 28% of the patients were still on anti-epileptic drugs and only 14% had seizures in the last year. Residual symptoms reported by patients or relatives were mostly memory deficits, apathy and difficulties with spatial orientation. 86% suffered from persistent amnesia for the disease period. These patients did not remember visits and admissions to the hospital, and lacked memories of life events happening during

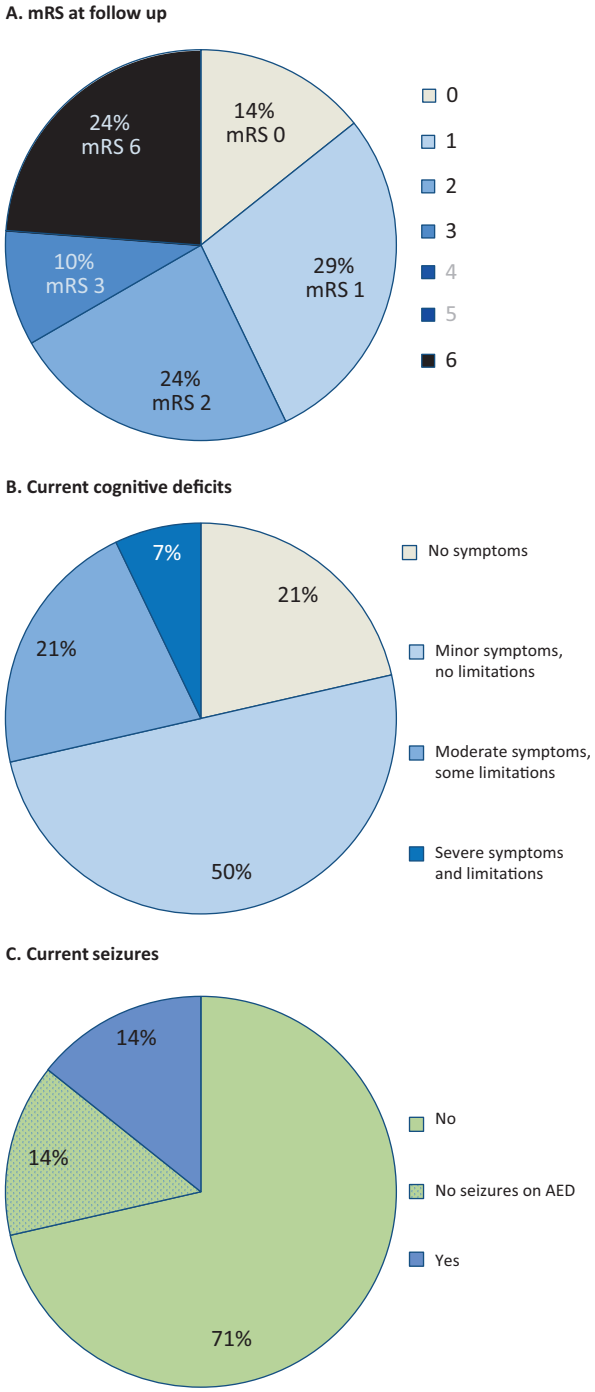

Figure 2. Outcome in patients with follow-up ≥ 2 years (n = 21). A. Modified Rankin Scale (mRS) at follow-up. Fourteen patients (67%) had a favorable outcome (mRS 0-2). Five patients had died (24%), of which four died within two years (2-year case fatality rate 19%). B. Current cognitive deficits, only including living patients without recent relapse. C. Seizures in the last 12 months, only including living patients without recent relapse. AED = anti-epileptic drugs.

disease. Retrograde amnesia was reported as well, often expressed as lack of memories of holidays. Three patients (21%) reported persistent insomnia, with sleep disorder confirmed with PSQI>5. No patient had increased ESS indicating daytime sleepiness. Overall, median time from symptom onset to maximum recovery was 67 weeks (IQR 50-115, range 36-269 weeks). There was no relation between treatment delay and time to maximum improvement or final mRS (p=0.76 and p=0.86 respectively).

Fifteen patients were eligible for neuropsychological assessment (follow-up ≥2 years), of which twelve agreed to participate. Assessment was performed after median follow-up of 44 months (range 25-95). Residual behavioral problems precluded reliable assessment in one patient (mRS=3), allowing analysis of eleven patients only, all mRS 0-2. Results for spatial recognition (SRM) were inferior to normative data (mean z-score -1.05, 95%-CI -1.89 to -0.23, p=0.018), while patients had normal scores on the other tests for visual memory. Patients had normal scores on the other primary outcome measure executive function (IED) and the secondary outcome measures. (Table 3)

Relapse rate was analyzed in the 17 patients with at least two years follow-up alive. Six patients (35%) had a relapse. Two patients were initially untreated and LGI1-antibodies were only tested at relapse. In four, relapses occurred despite being treated with immunotherapy in the acute phase. None of these patients used long-term immunosuppressive drugs when relapse occurred. In those tested (n=2), antibodies were absent in between the episodes and had reoccurred at relapse. Median time from onset of initial disease to relapse was 35 months (range 21-98).

Table 3: results of neuropsychological assessment performed with CANTAB

Test	Outcome measure	n	Z-score (mean, SD)	p-value	95% CI
Verbal memory					
Graded naming task (GNT)	Percent correct	11	0.21 (0.65)	0.30	-0.22 – 0.65
Visual memory					
Delayed matching to sample (DMS)	Percent correct (all delays)	10	0.07 (0.64)	0.75	-0.39 – 0.53
Pattern recognition memory (PRM)	Percent correct	11	0.06 (0.98)	0.85	-0.39 – 0.53
Spatial recognition memory (SRM)	*Percent correct*	*11*	*-1.06 (1.24)*	*0.018 **	*-1.89 – -0.23*
Executive function					
Intra-extra dimensional set shift (IED)	*Total errors*	*11*	*0.25 (0.68)*	*0.25*	*-0.21 – 0.70*
Spatial working memory (SWM)	Between errors	11	0.36 (1.14)	0.32	-0.40 – 1.13
Spatial span (SSP)	Span length	11	-0.46 (1.72)	0.40	-1.61 – 0.70
Attention					
Matching to sample (MTS)	Percent correct	11	-0.67 (0.95)	0.041 †	-1.31 - -0.03
Reaction time (RTI)	Mean simple reaction time	10	-0.48 (1.54)	0.36	-1.58 – 0.63

CANTAB = Cambridge Neuropsychological Test Automated Battery.
Cursive tests were upfront defined as primary outcome measures.
*p<0.025 (cut-off value for primary outcome measures, Bonferroni correction)
† p<0.05 (cut-off value for secondary outcome measure, uncorrected p-value should be considered carefully)

Discussion

We provide detailed clinical information of 38 patients with antibodies directed to LGI1 and report important incidence rates and long-term outcome. More insight in the course and semiology of seizures is essential to improve diagnosis. Seizures respond quickly to immunotherapy, but long-term follow-up showed cognitive improvement as well. Other important results of long-term follow-up were remarkable persistent amnesia for the disease period, a high relapse rate and the evolvement of MTS.

Annual incidence of anti-LGI1 encephalitis in the Netherlands was 0.83 per million, which is in the same order of magnitude as Creutzfeldt-Jakob disease[12] or Lambert-Eaton myasthenic syndrome.[13] The increase of the incidence of LGI1 diagnosis is probably due to better disease recognition, but underdiagnosis is still suspected.

The recognition of seizures is a clue for early diagnosis. 47% of the patients had FBDS, an early symptom comparable to 40-71% in other series.[14,15] Recognition of these short seizures might be complicated by the common absence of ictal EEG abnormalities, which has been reported before.[8] 66% of our patients had focal seizures with mainly dyscognitive or autonomic features, also early in disease course. A few cases presenting with pilomotor seizures have been reported before.[16] These cases also support our finding that focal seizures and FBDS were initially not recognized by treating physicians, including neurologists. Special attention should be paid to repeated attacks which patients describe as 'indefinable feeling', 'thoughts being pulled away' or autonomic features including goose bumps. Tonic-clonic seizures are more easily recognized, but usually occur later in disease course.

Almost 90% of surviving patients had a favorable outcome. Patients were left with persistent amnesia for the entire disease process and the preceding months or years. During interviews with patients and their relatives this symptom emerged to be very stressful to them, and was often misinterpreted as an expression of ongoing disease. Persistent amnesia for the period of disease has been recognized in anti-NMDA receptor encephalitis before[17] and was thought to be caused by disturbance of long-term potentiation (LTP), which is the key cellular mechanism in learning and memory.[17-19] A similar mechanism can be hypothesized in LGI1-encephalitis, because LGI1-ADAM22-AMPAR interaction is thought to influence both long-term depression (LTD) and LTP.[4,20] As LTD is essential for spatial memory as well,[21] disturbance of this process might be an explanation for spatial disorientation in LGI1-encephalitis. Neuropsychological assessment showed disturbed spatial recognition memory with normal performance on other memory tasks (in our limited sample size), implying a persistent disorder of spatial orientation, similarly as reported by the patients and their relatives. Apathy was also frequently reported, but was not tested formally by our computerized assessment.

Case fatality rate at two years was 19%. Another study reports 3/57 deaths (6%) after follow-up ranging from 2 to 60 months.[2] In our study, 2/5 deaths occurred in untreated patients, before LGI1-antibodies were recognized. Assuming immunotherapy is effective, better recognition and treatment of anti-LGI1 encephalitis is expected to decreases case fatality rate in the future.

Clinical relapses occurred more frequently than anticipated. In two patients, diagnosis was first made at relapse. Underdiagnoses of patients with a single disease episode might result in an overestimation of the relapse rate, similarly as reported in anti-NMDAR[22,23] and anti-AMPAR encephalitis.[24,25] Earlier series report relapses in 0-18% of the anti-LGI1 patients, but follow-up was shorter, whereas relapses tend to occur years after the initial disease episode.[2,14,26]

Patients with MTS had frequent seizures during disease course. MTS might be caused by seizure activity, or directly by inflammation. The development of MTS after limbic encephalitis was reported before in anti-LGI1 patients.[27] Similar hippocampal changes are reported after anti-GAD65 or anti-NMDAR encephalitis.[28,29]

Diagnosis of LGI1-antibodies can be complicated by low sensitivity of the CBA with CSF. Only 53% of the samples tested positive, resulting in significant delays in two patients until serum was tested. 6/8 CBA-negative CSF samples showed typical LGI1-antibody staining on rat hippocampal tissue, indicating that antibodies are actually present in the CSF. The need for serum analysis to detect LGI1-antibodies is just opposite from anti-NMDAR,[30] anti-GABAbR[31] or anti-AMPAR encephalitis.[24]
Median time from onset to recovery was more than a year, and time to relapse was almost three years. This underlines the need for long follow-up time in studies assessing outcome. To allow the inclusion of a considerable number of patients, this study was conducted retrospectively, with the associated limitations. We obtained most reliable data by analyzing patients' files and interviewing patients, relatives and treating physicians. All MRI's were blindly reviewed by one specialized neuroradiologist. However, variable scan protocols and lack of coronal images in some patients limited analyses.

Recognizing the clearly defined clinical syndrome of anti-LGI1 encephalitis is essential for early treatment. Disease course can be relatively slow, resembling dementia, and attention should be paid to seizures with subtle manifestations early in disease course. Long-term outcome of surviving patients is mostly favorable, although persistent amnesia for the disease period is disturbing for patients. Relapses are common and physicians should be aware that these can occur up to years after the initial disease episode.

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Supplemental material

Seizure and EEG description of two patients with anti-LGI1 encephalitis

Patient 1 presents with painful legs and hyperhidrosis. After six months paroxysmal symptoms started, consisting of faciobrachial dystonic seizures and focal seizures with dyscognitive and autonomic features, as described below. Progressive cognitive impairment evolved, followed by tonic-clonic seizures. Two different attacks occurred during 24-hours EEG:

- Up to seventy attacks lasting for several seconds with a tonic posture of the right hand, arm, neck and the right corner of the mouth. Patient is able to push the button to report these events. Ictal EEG recordings show no abnormalities besides muscle artifacts.
- Up to twenty episodes characterized by pupillary dilation, heavy breathing and an anxious facial expression. The patient reports nausea and an urge to vomit. Consciousness seems to be retained and after the attack patient immediately responds appropriately. During the attack, EEG shows theta or delta activity, followed by sharp waves and spike and wave complexes over the midfrontal and left frontal areas, with rapid recovery.

Patient 2 presents with mild behavioral problems. A few months later, memory declined and seizures started. Patient reports autonomic attacks lasting only seconds, occurring several times a day. These attacks consist of goose bumps and a shiver, sometimes accompanied by smelling a strange odor. Spouse described an early attack with dyscognitive features while the patient was driving a car. Patient was told to stop the car and they changed seats. After a few minutes, patient asked 'how did I get in the passenger's seat?'. Forty attacks similar occurred during 24-hours EEG registration:

- Attacks start with a shiver, sniffing and a pale face. Patient stops talking but is able to follow commands, without remember these afterwards. EEG started to become slow and irregular, followed by sharp waves and spike and wave complexes with a maximum over the frontal-temporal areas.

Table e-1. Description of CANTAB tests included in neuropsychological assessment

Test	Cognitive domain	Description
Graded naming task (GNT)	Verbal memory	Objects appear on the screen. Participants are instructed to name the object.
Delayed matching to sample (DMS)	Visual memory	A complex pattern appears on the screen. After a brief delay, four patterns appear. Participants must touch the pattern that matches the sample.
Pattern recognition memory (PRM)	Visual memory	Learning phase: several patterns appear, one at a time. Recognition phase: participants choose which of two patterns they have seen before
Spatial recognition memory (SRM)*	Visual memory	Learning phase: a white square is shown in various locations. Recognition phase: participants choose which of two boxes is in a location previously presenting a square.
Intra-extra dimensional set shift (IED)*	Executive function	Participants must first use feedback to learn a rule involving two dimensions. When feedback implies that the rule has changed, the participant must shift attention to the previously irrelevant dimension. Derived from the Wisconsin Card Sorting Test.
Spatial working memory (SWM)	Executive function	Participants search for blue tokens by touching colored boxes throughout the screen, without returning to a box where a blue token was previously found.
Spatial span (SSP)	Executive function	White squares on the screen briefly change color in a variable sequence. Participants must remember the sequence and touch squares in the same order, with growing sequence length throughout the test.
Matching to sample (MTS)	Attention	A test pattern is shown. Participants have to choose the matching pattern from a possible 8 patterns, measuring speed and accuracy.
Reaction time (RTI)	Attention	The participant must hold down a button until a yellow spot appears on the screen, and then touch the yellow spot.

CANTAB = Cambridge Neuropsychological Test Automated Battery
*Primary outcome measures

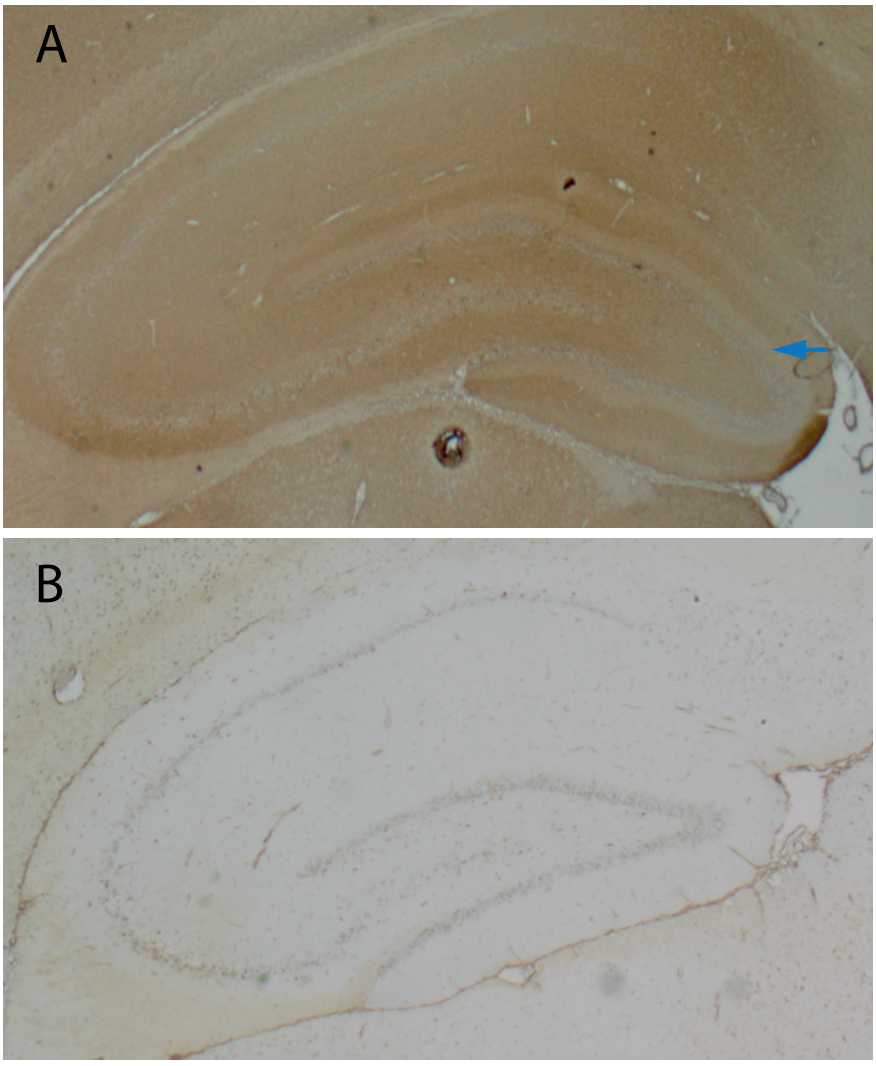

Figure e-1. Immunohistochemistry on rat brain. A) LGI1-antibody staining pattern. The hippocampus is stained, including the hilus and the outer 2/3rd of the dentate gyrus. Lack of staining of the inner 1/3rd of the dentate gyrus results in a pale line (blue arrow). B) Negative sample.

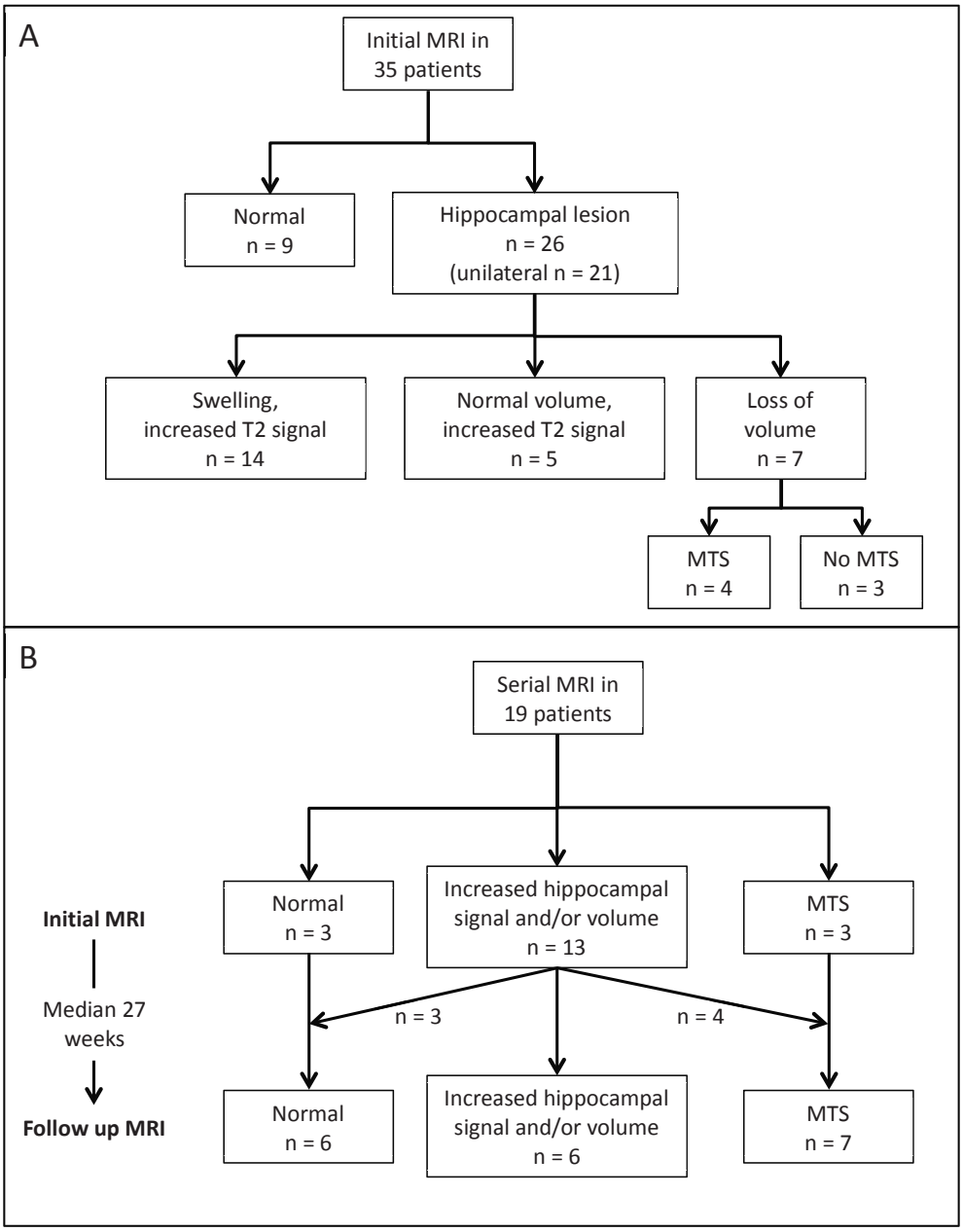

Figure e-2. A. Revision of initial MRI brain in 35 patients. B. Revision of serial MRI brain in 19 patients. MTS = mesotemporal sclerosis.

CHAPTER 3

Anti-LGI1 encephalitis is strongly associated with HLA-DR7 and HLA-DRB4

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Abstract

Leucine-rich glioma-inactivated1 (LGI1)-encephalitis is an antibody-associated inflammation of the limbic area. An autoimmune etiology is suspected but not proven yet. We performed HLA-analysis in 25 non-tumor anti-LGI1 patients and discovered a remarkably strong HLA-association. HLA-DR7 was present in 88% compared to 19.6% in healthy controls ($p=4.1*10^{-11}$). HLA-DRB4 was present in all patients and in 46.5% controls ($p=1.19*10^{-7}$). These findings support the autoimmune hypothesis. An exploratory analysis was performed in a small group of four tumor-LGI1 patients. The strong HLA association seems not applicable in these patients. Therefore, the absence of HLA-DR7 or HLA-DRB4 could raise tumor suspicion in anti-LGI1 patients.

Introduction

Leucine-rich glioma-inactivated1 (LGI1)-encephalitis is an antibody associated inflammation of the limbic area of the brain. Approximately 300 patients have been reported and annual incidence is 0.83 per million.[1] Patients exhibit a subacute onset of frequent short seizures, tonic clonic seizures and disturbance in memory and behavior. Tumors are present in 5-10% of the patients,[1-3] most commonly thymoma.

LGI1-antibodies are specific and thought to be pathogenic, although this has not been proven by passive transfer, as required according to the adapted Koch-Witebsky postulates.[4] Anti-LGI1 encephalitis shows resemblance to the phenotype linked to genetic disruption of LGI1 proteins, which serves as indirect evidence of autoimmunity. Circumstantial evidence is provided by the favorable response to immunotherapy in observational studies. A human leukocyte antigen (HLA) association would further support the autoimmune hypothesis.[4] HLA encodes for the major histocompatibility complex (MHC). MHC-class I restricted recognition by CD8+ T-cells leads mainly to a cytotoxic T-cell response, while MHC-class II restricted recognition by CD4+ T-cells often leads to a B-cell immune response. As the antibodies in anti-LGI1 encephalitis are considered pathogenic, a B-cell mediated immune disease is suspected. Therefore, a possible HLA-association is most likely to involve HLA-DR or HLA-DQ, encoding MHC-class II.

Specific HLA alleles have been linked to predisposition for autoimmune diseases, like myasthenia gravis (MG)[5], multiple sclerosis (MS)[6] and Lambert-Eaton myasthenic syndrome (LEMS).[7] Interestingly, the association of LEMS with HLA-B8-DR3-DQ2 was only found in patients without a tumor, suggesting a distinct immunopathogenetic route for paraneoplastic LEMS.[7] An HLA-association with antibody-mediated encephalitis has not been reported before.

In the clinical setting, we noticed a common HLA-DRB1*07 (DR7) allele in our anti-LGI1 patients. For systematic analysis, we performed HLA-phenotyping in a larger group of anti-LGI1 patients. The aim was to analyze the association between HLA-type and predisposition for anti-LGI1 encephalitis in non-tumor patients.

Methods

The study was performed in unrelated Dutch anti-LGI1 encephalitis patients from Caucasian descent. LGI1-antibodies were detected in serum, using a commercially available cell-based immunofluorescence assay (CBA)(Euroimmun, Lübeck, Germany) and confirmed with immunohistochemistry (IHC) on rat brain, as reported before.[8] Tumor screening had been performed with thoracic and abdominal CT or whole body FDG-PET, in line with recommendations.[9] The frequency of the HLA-alleles in the study group was compared to the frequency in 5,604 healthy Caucasian Dutch blood donors.[10]

The study was approved by the Institutional Review Board of the Erasmus Medical Center, Rotterdam. Patients were included in the study after their informed consent.

HLA analysis

HLA-class I typing was performed by the reverse SSO method on a suspension array platform using microspheres as a solid support to immobilize oligonucleotide probes (Luminex bead technology Immunocor Transplant Diagnostics Inc. Stamford, CT, USA). HLA-class II (DRB1, DQB1, DRB3/4/5) was genotyped using the sequence-specific oligonucleotide probe (PCR/SSOP) technique.[11] This results in medium-low resolution subtyping. The HLA-types are given as serotypes, according to the most common nomenclature. The serotypes are the protein products of various gene products. For example, the HLA-DR7 serotype comprises the gene products of HLA DRB1*07:01 to DRB1*07:05.

Tissue analysis

Formalin-fixed paraffin-embedded (FFPE) tumor tissue of a mesothelioma, resected from a patient with anti-LGI1 encephalitis, was analyzed for the presence of LGI1 proteins. 4 μm thick sections were stained with the Ventana Benchmark Ultra automated staining system (Ventana Medical System, Tuscon, AZ, USA). Briefly, after deparaffination the sectioned specimens were processed for 64 minutes antigen retrieval using Cell Conditioning Solution (CC1 Ventana-Ref.:950-124). After 32 minutes incubation with the primary antibody (LGI1, ab30868, Abcam, Massachusetts, USA. Dilution 1:250) at 360C, detection was performed using OptiView DAB IHC Detection Kit (760-700) after amplification with OptiView Amplification Kit (Ventana-ref.:760-099). The sections were counterstained with hematoxylin II (Ventana-Ref.:790-2208).

Statistical analysis

Differences in phenotype frequencies were analyzed with Fisher's Exact-Test. P-values were corrected for multiple testing (Sidak's method) and standardized for sample size disparity between the patient and control group (Good's method, adjusted to 1:3 ratio). $P_{corrected} < 0.05$ was considered significant. Odds ratios (OR) were calculated using Haldane's modification of Woolf's method. The main study group consisted of patients without a tumor. Analysis of HLA-subtype in anti-LGI1 patients with a tumor was exploratory.

Table 1: Patient characteristics and HLA-results.

Case	Gender	Age	Clinical subtype	Tumor	FU (mo)	DRB3,4,5	DRB1	DQB1
1	F	29	LE	No	7	DRB4	DRB1*08 DRB1*09	DQB1*03 DQB1*03
2	M	43	LE	No	6	DRB4	DRB1*01 DRB1*04	DQB1*03 DQB1*05
3	M	49	LE	No	4	DRB4 DRB3	DRB1*07 DRB1*12	DQB1*02 DQB1*03
4	F	51	LE	No	14	DRB4	DRB1*07 DRB1*04	DQB1*03 DQB1*03
5	M	54	LE	No	9	DRB4 DRB3	DRB1*07 DRB1*03	DQB1*02 DQB1*02
6	M	58	LE	No	15	DRB4 DRB5	DRB1*07 DRB1*15	DQB1*03 DQB1*06
7	F	58	LE	No	81	DRB4	DRB1*07 DRB1*01	DQB1*02 DQB1*05
8	M	59	LE	No	1	DRB4	DRB1*07 DRB1*04	DQB1*02 DQB1*03
9	F	60	MoS	No	95	DRB4	DRB1*07 DRB1*04	DQB1*03 DQB1*02
10	F	60	LE	No	42	DRB4	DRB1*07 DRB1*04	DQB1*02 DQB1*03
11	M	61	LE	No	79	DRB4	DRB1*07 BRB1*04	DQB1*02 DQB1*03
12	M	62	MoS	No	0	DRB4 DRB5	DRB1*07 DRB1*15	DQB1*02 DQB1*06
13	F	62	LE	No	6	DRB4 DRB3	DRB1*07 DRB1*03	DQB1*02 DQB1*03
14	M	63	LE	No	9	DRB4 DRB3	DRB1*07 DRB1*12	DQB1*02 DQB1*03
15	M	64	LE	No	0	DRB4	DRB1*07 DRB1*07	DQB1*02 DQB1*02
16	F	64	LE	No	85	DRB4	DRB1*07 DRB1*07	DQB1*02 DQB1*02
17	M	66	LE	No	4	DRB3 DRB4	DRB1*07 DRB1*11	DQB1*02 DQB1*03
18	M	68	LE	No	81	DRB4 DRB5	DRB1*07 DRB1*15	DQB1*02 DQB1*06
19	M	69	LE	No	26	DRB4	DRB1*07 DRB1*01	DQB1*02 DQB1*05
20	M	69	LE	No	8	DRB4	DRB1*07 DRB1*04	DQB1*02 DQB1*03
21	M	72	LE	No	9	DRB4	DRB1*07 DRB1*01	DQB1*02 DQB1*05
22	M	72	LE	No	18	DRB4	DRB1*07 DRB1*01	DQB1*02 DQB1*05
23	F	77	LE	No	73	DRB4	DRB1*07 DRB1*07	DQB1*02 DQB1*03
24	M	78	LE	No	18	DRB5 DRB5	DRB1*09 DRB1*16	DQB1*03 DQB1*05
25	M	80	LE	No	28	DRB4	DRB1*07 DRB1*04	DQB1*02 DQB1*03
26	M	60	LE	Rectum carcinoma (2 mo before)	79	DRB4	DRB1*07 DRB1*04	DQB1*02 DQB1*03
27	M	60	MoS	Neuroendocrine pancreas tumor (9 mo before)	34	DRB4	DRB1*07 DRB1*01	DQB1*02 DQB1*05
28	F	66	LE	Thymoma (2 mo after)	3	DRB3	DRB1*11 DRB1*13	DQB1*03 DQB1*06
29	M	67	LE	Mesothelioma (At relapse)	72	DRB4 DRB5	DRB1*04 DRB1*15	DQB1*03 DQB1*06

FU = follow up. Mo = months. LE = limbic encephalitis. MoS = Morvan's syndrome

Results

25 non-tumor and 4 tumor patients were included, 17/25 (68%) and 3/4 (75%) were male, respectively. Median age at disease onset was 62 years (IQR 58.5-68.5, range 29-80). 26 patients had a limbic encephalitis (LE). Three patients had LE with additional features, fulfilling criteria for Morvan's syndrome. In the course of disease, all patients had memory deficits and 25 patients (86%) had seizures. Median follow up was 15 months. (Table 1)

Non-tumor anti-LGI1 patients vs healthy controls

HLA-DR7 allele was present in 22/25 non-tumor anti-LGI1 patients (88%) and in 1,098/5,604 (19.6%) healthy controls (OR 26.37, 95%-CI 8.54–81.49, $p_{corrected}=4.1*10^{-11}$). Three anti-LGI1 patients (12%) were homozygous for HLA-DR7, compared to 1.0% in the control population (p=0.0021). The three HLA-DR7 negative patients did not differ from HLA-DR7 positive patients with regard to age and gender (p=0.84 and p=1.00, respectively) and they all presented with typical features of anti-LGI1 encephalitis.[1]

HLA-DR7 is known be linked to DRB4, which was present in all 25 patients, compared to 46.5% of the controls (OR 58.59, 95%-CI 3.57–962.84, $p_{corrected}=1.19*10^{-7}$). The high incidence of DQ2 is explained by its link with DR7. No positive association with other class I or class II HLA alleles was found. (Table 2 and Supplementary Table)

Table 2: The frequency of HLA-alleles in non-tumor anti-LGI1 patients and controls.

	Cases			Controls			Woolf-Haldane	95% C.I.		Fishers Exact Test (2-sided)		
	pos	neg	%	pos	neg	%	OR	lower	upper	P	Pc	Pc2
B*13	6	19	24,0	229	5375	4,1	7,81	3,182	19,155	0,0005	0,0179	0,1340
DRB1*07	22	3	88,0	1098	4506	19,6	26,37	8,535	81,489	0,0000	0,0000	<0,0001*
DRB1*13	0	25	0,0	1393	4211	24,9	0,06	0,004	0,974	0,0016	0,0222	0,1667
DRB3	5	20	20,0	3744	1860	66,8	0,13	0,052	0,342	0,0000	0,0000	0,0001*
DRB4	25	0	100,0	2608	2996	46,5	58,59	3,565	962,842	0,0000	0,0000	<0,0001*
DQB1*02	20	5	80,0	2204	3400	39,3	5,75	2,240	14,759	0,0000	0,0003	0,0024*
DQB1*06	3	22	12,0	2470	3134	44,1	0,20	0,064	0,609	0,0009	0,0064	0,0481*

The table shows the major overpresentation of DRB1*07 and DRB4 in anti-LGI1 patients. As a result, the frequency of DRB3 and DQB1*06 are significantly reduced. The high frequency of DQB1*02 in patients is probably due to its common linkage with DRB1*07. Within the DRB1*07 positive patients, DQB1*02 is present in 91%, compared to 74% in the DQB1*07 positive controls (p = 0.0857). CI = confidence interval; neg = negative; OR = odds ratio; pos = positive.

Tumor anti-LGI1 patients

The DR7 allele was present in 2/4 tumor patients and 3 were DRB4 positive. One patient was negative for DR7 and DRB4 and had a thymoma detected 2 months earlier. Recently, tissue analyses confirmed the presence of LGI1 proteins in a LGI1-patient's thymoma.[12] The other DR7 negative patient had a mesothelioma, detected with screening at relapse (no tumor screening at initial disease episode). Tissue analysis confirmed the presence of LGI1-protein in the patient's mesothelioma (Fig 1). The two DR7 positive patients had a rectum carcinoma in situ and a neuroendocrine pancreas tumor, of which the relation with encephalitis remains questionable.

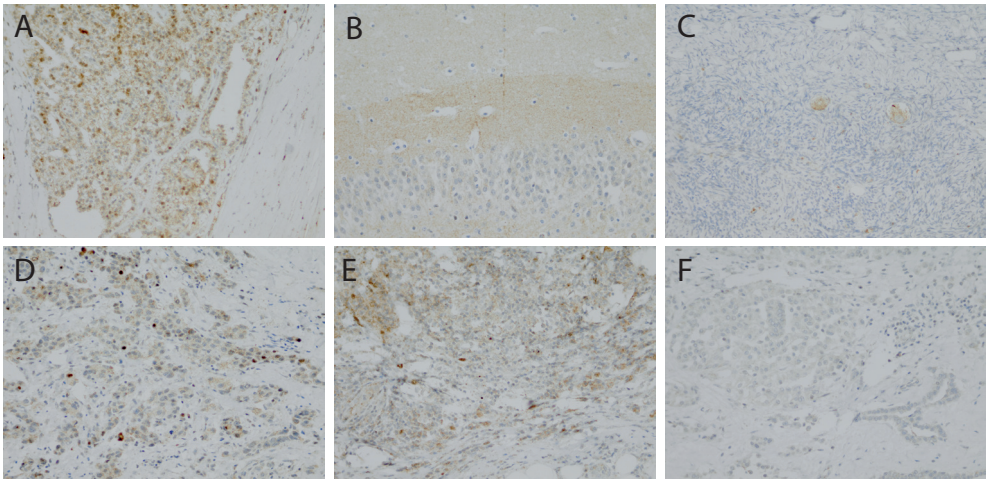

Figure 1. Tissue staining showing the presence of LGI1-protein in patient's mesothelioma.
A. Mesothelioma tissue from a patient with anti-LGI1-encephalitis. The positive staining with commercial LGI1 antibody (brown color) confirms the presence of LGI1 protein in the tumor. B. Hippocampal tissue, positive control. C. Ovarium tissue, negative control. D-F. Mesothelioma tissue from patients without LGI1 antibodies. Some mesothelioma contain LGI1 protein, showing positive staining (D, E), while others lack LGI1 protein (F). Figures are at x200 magnification.

Discussion

We report a remarkably strong association of non-tumor anti-LGI1 encephalitis with HLA-DR7 and HLA-DRB4. This is the first report of a genetic predisposition for antibody-mediated limbic encephalitis and supports the autoimmune hypothesis. The strong HLA association seems not to apply to the small group of four tumor patients, suggesting two different immunopathogenic pathways. Considering the small size, absence of HLA-DR7 or HLA-DRB4 in anti-LGI1 patients could raise tumor suspicion. In non-tumor anti-LGI1 patients, HLA-DRB4 was present in 100% and HLA-DR7 in 88%, comparable to the strong association of HLA-B27 in patients with ankylosing spondylitis[13]. HLA-DR53 (the molecule encoded by HLA-DRB4) is associated with several diseases, such as Crohn's disease,[14] rheumatoid arthritis,[15] and celiac disease.[16]

Little is known about DR7 associations. Limited data suggest an association with polyarthritis in psoriasis patients.[17] Functional studies are needed to establish whether DRB4 or DR7 is responsible for antigen presentation. Our results enable further studies, including the possibility to culture disease-specific T-cells from patient's peripheral blood. In addition, the target epitopes for LGI1-antibodies can possibly be detected by analyzing which fragment of LGI1-protein fits to the unique characteristics of the involved MHC-II molecule.

LGI1-antibodies are mainly IgG4 subclass antibodies.[18] An HLA-association was earlier described in IgG4-mediated diseases: MuSK-antibody mediated MG and pemphigus vulgaris are both associated with HLA-DR14-DQ5 and IgLON5 is associated with DR1-DQ5.[19,20] Anti-LGI1 encephalitis is the first IgG4-associated disease with a different HLA-type than DQ5.

Contrary to non-tumor-LGI1 patients, the strong HLA associations were not seen in the four anti-LGI1 patients with a tumor. This suggests two separate immunopathogenic routes leading to one disease, as described in LEMS before. HLA-B8-DR3 was only linked to non-tumor LEMS, while no HLA-association was found in LEMS associated to small-cell lung cancer.[7] In both LEMS and anti-LGI1 encephalitis, a direct autoimmune etiology is supported by the HLA-link in non-tumor patients, while a paraneoplastic etiology is likely in tumor patients. Our results support the latter by the demonstration of LGI1-proteins in a patient's tumor. This suggests that the tumor directly triggers the immune response, similar to LEMS.[21] Considering our small sample size we cannot draw firm conclusion in anti-LGI1 encephalitis, but the absence of HLA-DR7 or DRB4 seems to increase the probability of a tumor, and should lead to extensive screening.[9] Importantly, the presence of HLA-DR7 and DRB4 is insufficient to exclude a tumor in anti-LGI1-patients.

This study has some limitations. First, follow up in most patients was less than the four years needed to exclude a tumor.[9] However, the current HLA-DRB4 and DR7 associations are remarkably strong. Detecting a tumor in coming years will only slightly change the strength of these associations. Our study was limited by small sample size, especially of anti-LGI1 patients with a tumor, and confirmation of our results is mandatory.

Non-tumor anti-LGI1 encephalitis is strongly associated with the presence of HLA-DR7 and HLA-DRB4, underlining the autoimmune hypothesis. Our HLA results and the results of tumor tissue analysis suggest an alternative immunopathogenetic route in tumor patients. We recommend HLA subtyping in anti-LGI1 patients. If HLA-DR7 or DRB4 is absent, extended tumor search including follow up is indicated.

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Supplemental material

Supplementary Table: The frequency of all HLA-alleles in non-tumor anti-LGI1 patients and controls

	Cases			Controls			Woolf-Haldane	95% C.I.		Fishers Exact Test (2-sided)		
	pos	neg	%	pos	neg	%	OR	lower	upper	P	Pc	Pc2
A*01	6	19	24,0	1887	3717	33,7	0,66	0,270	1,598	0,3979	1,0000	0,5000
A*02	14	11	56,0	2745	2859	49,0	1,31	0,605	2,853	0,5503	1,0000	0,5000
A*03	8	17	32,0	1640	3964	29,3	1,17	0,516	2,669	0,8260	1,0000	0,5000
A*11	1	24	4,0	649	4955	11,6	0,47	0,090	2,434	0,3519	0,9998	0,5000
A*23	1	24	4,0	134	5470	2,4	2,49	0,475	13,063	0,4557	1,0000	0,5000
A*24	2	23	8,0	921	4683	16,4	0,54	0,147	1,995	0,4138	1,0000	0,5000
A*25	0	25	0,0	122	5482	2,2	0,88	0,053	14,498	1,0000	1,0000	0,5000
A*26	2	23	8,0	248	5356	4,4	2,29	0,619	8,499	0,3057	0,9993	0,5000
A*29	3	22	12,0	299	5305	5,3	2,76	0,888	8,549	0,1477	0,9591	0,5000
A*30	2	23	8,0	276	5328	4,9	2,05	0,553	7,594	0,3521	0,9998	0,5000
A*31	3	22	12,0	314	5290	5,6	2,62	0,844	8,116	0,1637	0,9720	0,5000
A*32	2	23	8,0	355	5249	6,3	1,57	0,425	5,812	0,6707	1,0000	0,5000
A*33	0	25	0,0	113	5491	2,0	0,95	0,057	15,680	1,0000	1,0000	0,5000
A*34	0	25	0,0	17	5587	0,3	6,26	0,367	106,936	1,0000	1,0000	0,5000
A*36	0	25	0,0	9	5595	0,2	11,55	0,655	203,762	1,0000	1,0000	0,5000
A*66	0	25	0,0	33	5571	0,6	3,26	0,194	54,679	1,0000	1,0000	0,5000
A*68	1	24	4,0	517	5087	9,2	0,60	0,115	3,137	0,7237	1,0000	0,5000
A*69	0	25	0,0	9	5595	0,2	11,55	0,655	203,762	1,0000	1,0000	0,5000
A*74	0	25	0,0	5	5599	0,1	19,96	1,075	370,551	1,0000	1,0000	0,5000
A*80	0	25	0,0	2	5602	0,0	43,94	2,058	938,319	1,0000	1,0000	0,5000
B*07	4	21	16,0	1391	4213	24,8	0,63	0,229	1,754	0,3633	1,0000	0,5000
B*08	5	20	20,0	1436	4168	25,6	0,78	0,303	1,999	0,6493	1,0000	0,5000
B*13	6	19	24,0	229	5375	4,1	7,81	3,182	19,155	0,0005	0,0179	0,1340
B*14	1	24	4,0	217	5387	3,9	1,52	0,290	7,929	1,0000	1,0000	0,5000
B62	8	17	32,0	837	4732	15,0	2,74	1,206	6,249	0,0427	0,8254	0,5000
B63	0	25	0,0	44	5525	0,8	2,43	0,146	40,618	1,0000	1,0000	0,5000
B75	0	25	0,0	16	5553	0,3	6,60	0,385	112,991	1,0000	1,0000	0,5000
B76	0	25	0,0	1	5568	0,0	72,79	2,896	1829,526	1,0000	1,0000	0,5000
B77	0	25	0,0	9	5560	0,2	11,48	0,650	202,487	1,0000	1,0000	0,5000
B*18	1	24	4,0	414	5190	7,4	0,77	0,147	3,999	1,0000	1,0000	0,5000
B*27	1	24	4,0	405	5199	7,2	0,79	0,151	4,095	1,0000	1,0000	0,5000
B*35	4	21	16,0	1090	4514	19,5	0,87	0,313	2,399	0,8040	1,0000	0,5000
B*37	0	25	0,0	181	5423	3,2	0,59	0,036	9,662	1,0000	1,0000	0,5000

B*38	0	25	0,0	186	5418	3,3	0,57	0,035	9,394	1,0000	1,0000	0,5000
B*39	2	23	8,0	229	5375	4,1	2,49	0,672	9,240	0,2739	1,0000	0,5000
B60	0	25	0,0	676	4832	12,3	0,14	0,009	2,303	0,0644	0,9303	0,5000
B61	1	24	4,0	198	5310	3,6	1,64	0,313	8,569	0,6006	1,0000	0,5000
B*41	2	23	8,0	86	5518	1,5	6,79	1,811	25,434	0,0575	0,9062	0,5000
B*42	0	25	0,0	9	5595	0,2	11,55	0,655	203,762	1,0000	1,0000	0,5000
B*44	8	17	32,0	1257	4347	22,4	1,68	0,738	3,820	0,2378	1,0000	0,5000
B*45	0	25	0,0	60	5544	1,1	1,80	0,108	29,858	1,0000	1,0000	0,5000
B*46	0	25	0,0	9	5595	0,2	11,55	0,655	203,762	1,0000	1,0000	0,5000
B*47	0	25	0,0	29	5575	0,5	3,71	0,220	62,309	1,0000	1,0000	0,5000
B*48	0	25	0,0	5	5599	0,1	19,96	1,075	370,551	1,0000	1,0000	0,5000
B*49	1	24	4,0	84	5520	1,5	4,00	0,759	21,090	0,3170	1,0000	0,5000
B*50	0	25	0,0	84	5520	1,5	1,28	0,077	21,217	1,0000	1,0000	0,5000
B*51	2	23	8,0	590	5014	10,5	0,90	0,245	3,337	1,0000	1,0000	0,5000
B*52	0	25	0,0	50	5554	0,9	2,16	0,130	35,915	1,0000	1,0000	0,5000
B*53	0	25	0,0	40	5564	0,7	2,69	0,161	45,013	1,0000	1,0000	0,5000
B*54	0	25	0,0	2	5602	0,0	43,94	2,058	938,319	1,0000	1,0000	0,5000
B*55	0	25	0,0	239	5365	4,3	0,44	0,027	7,237	0,6245	1,0000	0,5000
B*56	0	25	0,0	77	5527	1,4	1,40	0,084	23,179	1,0000	1,0000	0,5000
B*57	2	23	8,0	368	5236	6,6	1,51	0,409	5,592	0,6783	1,0000	0,5000
B*58	0	25	0,0	93	5511	1,7	1,16	0,070	19,128	1,0000	1,0000	0,5000
B*67	0	25	0,0	1	5603	0,0	73,25	2,914	1841,024	1,0000	1,0000	0,5000
B*71	0	25	0,0	19	5550	0,3	5,58	0,328	94,955	1,0000	1,0000	0,5000
B72	0	25	0,0	26	5543	0,5	4,10	0,243	69,146	1,0000	1,0000	0,5000
B*73	0	25	0,0	2	5602	0,0	43,94	2,058	938,319	1,0000	1,0000	0,5000
B*78	0	25	0,0	3	5601	0,1	31,38	1,580	623,201	1,0000	1,0000	0,5000
B*81	0	25	0,0	3	5601	0,1	31,38	1,580	623,201	1,0000	1,0000	0,5000
C*01	2	23	8,0	323	5281	5,8	1,74	0,469	6,429	0,6532	1,0000	0,5000
C*02	3	22	12,0	575	5029	10,3	1,36	0,439	4,206	0,7379	1,0000	0,5000
C*03	7	18	28,0	1665	3939	29,7	0,96	0,410	2,244	1,0000	1,0000	0,5000
C*04	6	19	24,0	1287	4317	23,0	1,12	0,459	2,722	0,8159	1,0000	0,5000
C*05	1	24	4,0	764	4840	13,6	0,39	0,074	2,019	0,2408	0,9789	0,5000
C*06	8	17	32,0	898	4706	16,0	2,54	1,118	5,791	0,0492	0,5067	0,5000
C*07	14	11	56,0	3140	2464	56,0	0,99	0,456	2,149	1,0000	1,0000	0,5000
C*08	1	24	4,0	233	5371	4,2	1,41	0,269	7,362	1,0000	1,0000	0,5000
C*12	0	25	0,0	466	5138	8,3	0,22	0,013	3,554	0,2622	0,9858	0,5000
C*14	1	24	4,0	117	5487	2,1	2,86	0,544	15,018	0,4119	0,9994	0,5000
C*15	0	25	0,0	287	5317	5,1	0,36	0,022	5,972	0,6364	1,0000	0,5000

C*16	4	21	16,0	343	5261	6,1	3,21	1,154	8,908	0,0643	0,6059	0,5000
C*17	2	23	8,0	97	5507	1,7	6,01	1,607	22,477	0,0707	0,6418	0,5000
C*18	0	25	0,0	2	5602	0,0	43,94	2,058	938,319	1,0000	1,0000	0,5000
DRB1*01	5	20	20,0	1234	4370	22,0	0,95	0,370	2,440	1,0000	1,0000	0,5000
DR17	2	23	8,0	1524	3990	27,6	0,28	0,075	1,027	0,0250	0,2988	0,5000
DR18	0	25	0,0	8	5506	0,1	12,70	0,714	225,974	1,0000	1,0000	0,5000
DRB1*04	8	17	32,0	1557	4047	27,8	1,26	0,555	2,870	0,6563	1,0000	0,5000
DRB1*07	22	3	88,0	1098	4506	19,6	26,37	8,535	81,489	0,0000	0,0000	0,0000*
DRB1*08	1	24	4,0	361	5243	6,4	0,89	0,170	4,633	1,0000	1,0000	0,5000
DRB1*09	2	23	8,0	160	5444	2,9	3,61	0,971	13,419	0,1609	0,9142	0,5000
DRB1*10	0	25	0,0	132	5472	2,4	0,81	0,049	13,374	1,0000	1,0000	0,5000
DRB1*11	1	24	4,0	930	4674	16,6	0,31	0,059	1,602	0,1071	0,7951	0,5000
DRB1*12	2	23	8,0	210	5394	3,7	2,73	0,735	10,116	0,2422	0,9794	0,5000
DRB1*13	0	25	0,0	1393	4211	24,9	0,06	0,004	0,974	0,0016	0,0222	0,1667
DRB1*14	0	25	0,0	373	5231	6,7	0,27	0,017	4,520	0,4073	0,9993	0,5000
DRB1*15	3	22	12,0	1356	4248	24,2	0,49	0,158	1,505	0,2389	0,9781	0,5000
DRB1*16	1	24	4,0	165	5439	2,9	2,01	0,384	10,539	0,5276	1,0000	0,5000
DRB3	5	20	20,0	3744	1860	66,8	0,13	0,052	0,342	0,0000	0,0000	0,0001*
DRB4	25	0	100,0	2608	2996	46,5	58,59	3,565	962,842	0,0000	0,0000	0,0000*
DRB5	4	21	16,0	1508	4096	26,9	0,57	0,205	1,573	0,2645	0,6021	0,5000
DQB1*02	20	5	80,0	2204	3400	39,3	5,75	2,240	14,759	0,0000	0,0003	0,0024*†
DQ7	6	19	24,0	1641	3894	29,6	0,79	0,325	1,925	0,6632	0,9995	0,5000
DQ8	6	19	24,0	1073	4462	19,4	1,39	0,569	3,375	0,6105	0,9986	0,5000
DQ9	6	19	24,0	504	5031	9,1	3,32	1,362	8,115	0,0226	0,1480	0,5000
DQB1*04	0	25	0,0	340	5264	6,1	0,30	0,018	4,991	0,3993	0,9718	0,5000
DQB1*05	6	19	24,0	1840	3764	32,8	0,68	0,280	1,659	0,4008	0,9723	0,5000
DQB1*06	3	22	12,0	2470	3134	44,1	0,20	0,064	0,609	0,0009	0,0064	0,0481*

*$p_{corrected} < 0.05$. †The high frequence of DQB1*02 in patients is probably due to its linkage with DRB1*07. Within the DRB1*07 positive patients, DQB1*02 is present in 91%, compared to 74% in the DQB1*07 positive controls ($p = 0.0857$).

CHAPTER 4

The clinical spectrum of Caspr2-antibody associated disease

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Abstract

Objectives

To report a large cohort of patients with antibodies against contactin-associated protein-like 2 (Caspr2) and provide the clinical spectrum of this disorder.

Methods

Serum and CSF samples were assessed at two neuroimmunology centers in Barcelona and Rotterdam. Patients were included if Caspr2-antibodies were confirmed with two independent techniques, including brain immunohistochemistry and cell-based assay. Clinical information was obtained by the authors or provided by treating physicians after patients' informed consent.

Results

Median age at symptom onset was 66 years. 34/38 patients were male. Median time to nadir of disease was 4 months (in 30% >1 year). The most frequent syndromes included limbic encephalitis (42%) and Morvan's syndrome (29%). 77% of the patients had ≥3 of the following symptoms: encephalopathy (cognitive deficits/seizures), cerebellar dysfunction, peripheral nervous system hyperexcitability, dysautonomia, insomnia, neuropathic pain or weight loss. A tumor, mostly thymoma, occurred in 19% of the patients. IgG4 subclass antibodies were present in all patients, 63% also had IgG1 antibodies. Treatment-response occurred in 93% of the patients and 25% had clinical relapses.

Conclusions

Caspr2-antibodies associate with a treatable disorder that predominantly affects elderly men. The resulting syndrome may vary among patients but it usually includes a set of well-established symptoms. Recognition of this spectrum of symptoms and consideration of the protracted clinical course are important for early diagnosis of this disorder. Prompt immunotherapy and tumor therapy (if needed) often result in improvement.

Introduction

Contactin-associated protein-like 2 (Caspr2) is a membrane protein expressed in the central and peripheral nervous system. It is essential for proper localization of voltage-gated potassium channels (VGKC). Antibodies to VGKC were initially reported in patients with neuromyotonia, Morvan's syndrome and limbic encephalitis (LE).[1-3] However, while the clinical spectrum emerged, it became clear that the antibodies were not directed against the VGKC subunits but to associated proteins. Two of these proteins were identified in 2010: leucine-rich glioma-inactivated1 (LGI1) and Caspr2.[4, 5] Antibodies to LGI1 are mainly associated with LE and faciobrachial dystonic seizures, but the clinical spectrum of Caspr2-antibodies is more diverse. Most reports on Caspr2 autoimmunity consist of clinically pre-selected groups of patients with Morvan's syndrome[6], epilepsy[7] or pain syndromes.[8] In other reports, patients with Caspr2-antibodies were analyzed along with patients with antibodies to LGI1 or unknown proteins considered within the VGKC-complex.[9,10] Overall the clinical spectrum of Caspr2-autoimmunity remains not well defined. We report here the largest series of patients with Caspr2 antibodies and provide a framework for the clinical recognition of this disorder.

Methods

Patients

The study population consisted of patients suspected to have autoimmune or paraneoplastic neurological disorders whose serum or cerebrospinal fluid (CSF) were analyzed at two referral centers (Center of Experimental Neuroimmunology, Institut d'Investigacions Biomèdiques August Pi i Sunyer [IDIBAPS], Hospital Clinic, University of Barcelona, and Department of Immunology, Erasmus University Medical Center, Rotterdam) between 1994 and 2015. Patients with confirmed Caspr2-antibodies were included in the study. Serum and CSF (if available) were tested using brain immunohistochemistry (IHC) and cell-based assays (CBA) in parallel in both institutions. Patients were considered to have Caspr2-antibodies if both tests were positive in at least one of the samples. Clinical information was obtained from the treating physicians in a standardized fashion after patient's informed consent, or patients were seen by one of the authors (n = 15). Peripheral nerve hyperexcitability (PNH) was defined as spontaneous muscle overactivity (i.e. myokymia, fasciculations) identified by the treating neurologist during physical examination or with electrophysiological studies.[11] Morvan's syndrome was defined as a combination of a) cognitive symptoms or seizures, and b) peripheral nerve hyperexcitability and c) dysautonomia and/or insomnia. Limbic encephalitis was defined as an encephalitis with predominant clinical involvement of the limbic system (short-term memory loss, difficulty forming new memories, behavioral disorder) or MRI FLAIR/T2 abnormalities in the medial

temporal lobes. Pain was considered of neuropathic origin if it was described as 'burning' sensation, 'painful pins and needles' or had a compatible nerve distribution. Relapse was defined as reoccurrence of symptoms after full or partial recovery, with sustained improvement for at least two months.

Laboratory studies

The cell-based assays for determination of Caspr2[12] and other antibodies, brain tissue immunohistochemistry[13], radioimmunoassay to determine VGKC-complex antibodies, and immunoblot studies[14] have been previously reported, and are described in Supplemental material.

Statistical analysis

Fisher exact test was used for categorical data. Mann Whitney U was used for the comparison of continuous data.

Approval

The study was approved by the Institutional Review Boards of the University of Barcelona and the Erasmus University Medical Center, Rotterdam.

Results

Thirty-eight patients fulfilled the criteria of having Caspr2-antibodies confirmed with more than one test (Suppl. Figure e-1, Suppl. Table e-1); 10 additional cases had antibodies detected only with CBA but without confirmation with brain IHC (Suppl. Table e-2). The clinical features of patients with confirmed Caspr2-antibodies are shown in Table 1. Thirty-four of 38 (89%) were male; the median age at symptom onset was 66 years. Female patients were younger than male patients (median 49 vs 68 years, p = 0.008). The median time to nadir was four months; in 10/33 (30%) patients the nadir of the disease was reached ≥12 months after symptom onset. Morvan's syndrome was related to longer time to nadir than other syndromes (p=0.016).

Table 1. Patient characteristics and clinical features (n = 38)

Male gender	34/38 (89%)
Age in years (median; IQR; range)	66; 58-72; 25-77
Time to nadir in months (median; IQR; range)	4; 2.5-12; 0.2-42
Clinical syndrome - Limbic encephalitis - Morvan's syndrome - Peripheral nerve hyperexcitability syndrome - Cerebellar syndrome - Other	 - 16 (42%) (10/16 LE 'plus') - 11 (29%) - 5 (13%) - 3 (8%) - 3 (8%)
Presenting symptom - Cognitive disturbance - Epilepsy - Peripheral nerve hyperexcitability - Neuropathic pain - Cerebellar symptoms	 - 9/34 (26%) - 8/34 (24%) - 7/34 (21%) - 6/34 (18%) - 4/34 (12%)
Symptoms during course of disease	
Cognitive disturbance - Amnesia - Behavioral disorder - Hallucinations - Psychosis	30/38 (79%) - 24/35 (69%) - 21/33 (64%) - 10/30 (33%) - 6/32 (19%)
Epilepsy	19/36 (53%)
Peripheral nerve hyperexcitability	20/37 (54%)
Sleep disorder - Insomnia	19/28 (68%) 16/28 (57%)
Autonomic dysfunction, ≥ 1 of the following: - Hyperhydrosis - Tachycardia - Urinary retention or hesitation - Constipation - Orthostatic hypotension - Adie's pupil - Erectile dysfunction	14/32 (44%) - 7/14 (50%) - 4/14 (29%) - 3/14 (21%) - 2/14 (14%) - 2/14 (14%) - 2/14 (14%) - 1/14 (7%)
Weight loss	18/31 (58%)
Pain Subtype specified (n = 14) - Neuropathic - Muscles / joints	20/33 (61%) - 12/14 (86%) - 2/14 (14%)
Cerebellar symptoms	12/34 (35%)

Clinical phenotype

Sixteen patients (42%) developed LE. Ten of them had additional symptoms beyond the limbic system, such as cerebellar dysfunction or pain ('LE plus', 26%) and the other 6 had 'pure' LE (16%). Morvan's syndrome occurred in 11 (29%) patients, and PNH in 5 (13%). Two of these 5 patients also had insomnia, and another two had autonomic dysfunction. Three additional patients (8%) had predominant cerebellar symptoms, and the remaining 3 patients had a single seizure followed by pain syndrome (n = 1), painful polyneuropathy (n = 1) or mild amnestic syndrome with frontal lobe dysfunction (n = 1).

Most common presenting symptoms were cognitive disturbance (26%), seizures (24%), PNH (21%) or neuropathic pain (18%). During the course of disease, cognitive dysfunction was reported by the treating neurologist in 79% of the patients and 53% had seizures. In addition to cerebral symptoms, sleep disorder (68%), pain

(61%), weight loss (58%, median 10 kg), PNH (54%), autonomic dysfunction (44%) and cerebellar symptoms (35%) were common. The type of pain most frequently reported was neuropathic (86%), usually described as a burning sensation in the hands or feet; other types of pain included joint and muscle pain, thoracic pain, and lumbocoxalgia. The repertoire of seven symptoms comprise the spectrum of Caspr2 clinical manifestations (Table 2). In 77% of the patients ≥ 3 core symptoms were present and 61% had ≥ 4. Although mainly contributive to patient recognition, these symptoms possibly differentiate Caspr2-patients from patients with other antibodies. Among 35 LGI1 patients and 62 NMDAR patients, only 6 (17%) and 2 patients (3%) had ≥ 4 core symptoms (both $p < 0.001$), respectively.

Table 2. Caspr2 core symptoms and signs

Cerebral symptoms (cognition, epilepsy)
Cerebellar symptoms
Peripheral nerve hyperexcitability
Autonomic dysfunction
Insomnia
Neuropathic pain
Weight loss

Diagnostic tests

Cerebrospinal fluid was normal in 65% of the patients (Table 3). Seven patients had mild pleocytosis ranging from 6-20 cell/μL. Four of 6 patients with neuropathic pain had unremarkable nerve conduction studies. Needle EMG showed hyperexcitability in all patients with clinical features of PNH. In patients with cognitive decline and/or seizures, 70% had unremarkable MRI, while 24% showed bilateral T2 hyperintensity of the medial temporal lobes. One patient presenting with ataxia and dysarthria had an area of increased FLAIR/T2 signal in the brainstem. A patient with subacute cerebellar ataxia followed by limbic encephalitis had cerebellar atrophy on the MRI at presentation.

Caspr2-antibodies were detected in serum with both CBA and IHC in all patients. CSF brain IHC was negative in three patients, all with a tumor (and presenting with Morvan's syndrome). Possibly, CSF antibody titer was lower due to the predominant initial peripheral involvement in this syndrome and the presence of a clear systemic trigger of the immune response. Twenty eight serum samples were available for VGKC-complex RIA. The median titer was 414 pM; 25 patients (89%) had a positive result (titer >100 pM).

IgG subtype classification was available in 19 patients. All sera were IgG4 positive (100%) and twelve were also positive for IgG1 subclass antibodies (63%) (Fig 1). No clinical correlation with the presence of IgG1 antibodies was detected.

Table 3. Ancillary testing and laboratory results

Hyponatremia	4/31* (13%)
CSF - Normal - Cell count > 5 cell/µL - Protein > 0.58 g/L - Unmatched oligoclonal bands	 - 22/34 (65%) - 7/34 (21%) - 9/34 (26%) - 3/12 (25%)
EEG - Normal - Epileptic - Slow	 - 8/27 (30%) - 11/27 (41%) - 9/27 (33%)
EMG: PNP on nerve conduction studies - Patients with neuropathic pain	 2/6 (33%)
EMG: hyperexcitability on needle EMG - Patients with PNH symptoms - Patients without PNH symptoms	 - 15/15 (100%) - 1/8 (13%)
MRI brain (only patients with CNS symptoms) - Normal - Hyperintensity medial temporal lobes - Other abnormalities	 - 23/33 (70%) - 8/33 (24%; all bilateral) - 2/33 (6%)
Tumor	7/37 (19%)
Immunological testing	
Serum Caspr2 CBA positive	34/34 (100%)
Serum IHC positive (staining matching Caspr2 antibodies)	34/34 (100%)
CSF Caspr2 CBA positive	22/22 (100%)
CSF IHC positive (staining matching Caspr2 antibodies)	19/22 (86%)
LGI1 positivity	4/36 (11%)
VGKC RIA, picomol (n = 28). Median, range	414, 50-815
Immunoglobulin subtype - IgG1 - IgG4	 - 12/19 (63%) - 19/19 (100%)

*2/4 patients with hyponatremia had LGI1-antibodies in addition to their Caspr2-antibodies.

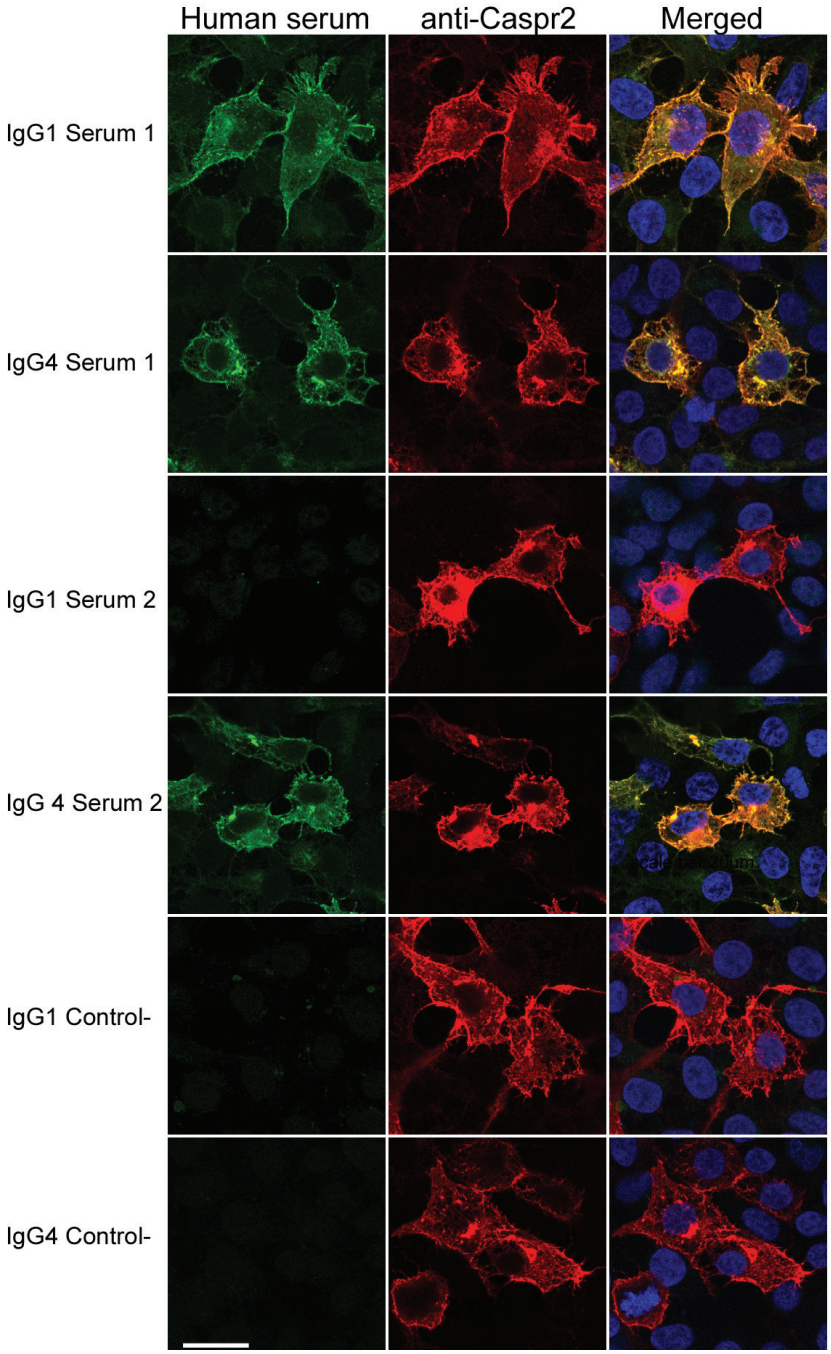

Figure 1: IgG subtyping by cell-based immunofluorescence assay.
Serum from patient 1 shows IgG1 and IgG4 reactivity with HEK cells expressing Caspr2. Serum from patient 2 shows only IgG4 reactivity. The control serum is from a healthy participant showing absence of IgG1 and IgG4 reactivity with Caspr2.

Co-morbidities

Seven (2 female, 5 male) of 37 (19%) patients had a tumor, including 4 thymoma, 1 adenocarcinoma of the lung, 1 carcinoma in situ of sigmoid, and 1 thoracic mass without pathological diagnosis (the patient died shortly after presenting neurological symptoms). Among the 4 patients with thymoma, 1 had tumor resection two months before onset of neurological symptoms, another had an unresectable thymoma that had been stable for several years, and two had a tumor relapse by the time of neurological disease onset. Interestingly, 6/7 tumor patients had PNH-syndrome with several additional 'core symptoms' or Morvan's syndrome, as compared to 10/30 non-tumor patients (uncorrected p = 0.029). Patients with a tumor had a similar progressive disease course as those without tumor (median time to peak of disease 3 versus 4.5 months, p = 0.72).

In addition to anti-Caspr2 associated symptoms, 3 patients (2 female) had myasthenia gravis (MG). Their anti-Caspr2-related syndromes included Morvan's syndrome (n = 2) and PNH syndrome (n = 1). Two of them had recurrent thymoma at the time of presentation and the third had thymic hyperplasia without thymoma.

Four patients (11%) had additional LGI1 antibodies: one had LE, one PNH syndrome, and two Morvan's syndrome. Co-occurrence of LGI1-antibodies was present in 2/7 tumor patients (both with thymoma) and in 2/30 non-tumor patients (p = 0.15). Serum sodium levels were available in three patients with LGI1-antibodies, two of them had hyponatremia. Caspr2-antibodies and LGI1-antibodies co-occurred in 2/4 female patients, but this was not different from male patients (2/32, p = 0.053).

Treatment and outcome

Twenty-eight of 30 patients without tumor were treated with immunotherapy, and the treatment effects in the first month were obtained in 23. The median delay between symptom onset and treatment was 6 months, ranging from 10 days to 9 years. Treatments included intravenous immunoglobulin (IVIg) only (n = 4), intravenous and/or oral steroids only (n = 7), plasma exchange only (n = 1), combination of IVIg and steroids (n = 7), combination of IVIg, steroids and plasma exchange (n = 2), or combination of steroids and plasma exchange (n = 2). Additionally, one patient was treated with azathioprine, and seven with second line immunotherapy (cyclophosphamide (n = 2) or rituximab (n = 5)). Full recovery was obtained in 9 patients (39%) and partial response in 12 (52%). Two patients (9%) did not respond to immunotherapy (Figure 2A). Six patients required repeated cycles of immunotherapy because symptoms progressed days to weeks after initial response (treatment related fluctuations). The various therapeutic strategies could not be compared, due to small numbers and selection.

Two patients were not treated; one of them had Morvan's syndrome and died before treatment could be started. The other patient was diagnosed recently. He had minor cognitive impairment and refused treatment.

Four of the 7 patients with a tumor were initially treated only with immunotherapy. Two showed transient improvement and the other two did not respond. However, all 4 patients had full neurological recovery after the tumor was identified and successfully treated with surgery or chemotherapy (Fig 2A). The effect of tumor treatment was unknown for the other 3 tumor patients.

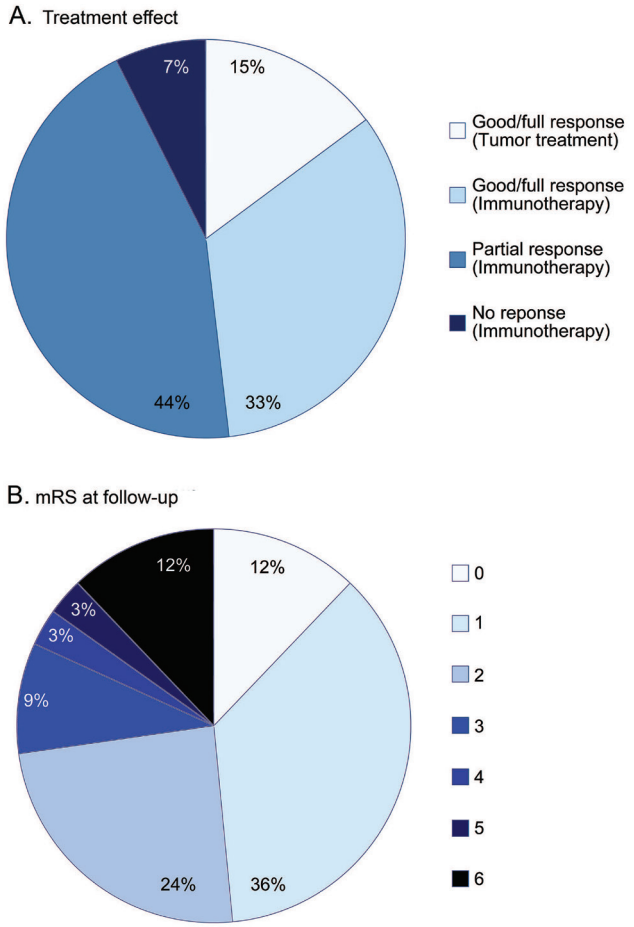

Figure 2: Treatment effect and outcome A. Effect of treatment in 27 patients. B. Modified Rankin Scale at follow-up in 33 patients. 0 - No symptoms. 1 - No significant disability. Able to carry out all usual activities, despite some symptoms. 2 - Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities. 3 - Moderate disability. Requires some help, but able to walk unassisted. 4 - Moderately severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted. 5 - Severe disability. Requires constant nursing care and attention, bedridden, incontinent. 6 - Dead.[26]

The median follow up was 36 months (range 3-168). Twenty-four of 33 patients (73%) had a favorable outcome at the last follow up (mRS ≤2)(Figure 2B). Four patients died: two died at initial stages of the neurological disease, and one during a relapse. The fourth patient died after four years in a nursing home (with serious cognitive residual symptoms and cardiac disease). Case fatality rate was 3% after one year and 10% after two years.

Seven of 28 (25%) patients with a ≥ 1 year follow up had clinical relapses. Relapses occurred in 3/3 initially untreated patients and 4/26 treated patients (p = 0.010), and presented 8 to 72 months after the initial episode (median 19 months; IQR 9 to 33). In 3 of these 7 patients the diagnosis of Caspr2 antibody-associated syndrome was during the relapse. At relapse, five patients had symptoms similar to those of the first episode, but the other two developed different core symptoms of the disease. A clarifying case is a man who presented at age 61 with visual hallucinations, behavioral problems, seizures and ataxia. Six year later he returned with PNH and dysarthria. The relapse rate did not differ between tumor and non-tumor patients (20% vs 25%, p = 1.00).

Discussion

We report 38 patients with Caspr2-antibodies. This is the largest and most detailed description of patients with this disorder and provides several relevant findings, 1) there is a well-defined spectrum of symptoms related to Caspr2-antibodies and most patients have symptoms affecting multiple areas of the nervous system, 2) the symptom development and course of the disease are often less rapid than those of other autoimmune encephalitis, 3) the disorder predominates in males, 4) approximately 25% of the patients had relapses, and 5) all patients had IgG4 subclass Caspr2-antibodies.

The diagnosis of this immune disorder can be complicated by the presentation with a combination of symptoms involving the central and peripheral nervous system. Although the clinical picture may vary among patients it usually includes a set of well-established symptoms. In 77% of the patients three or more core symptoms were present, including cognitive deficits/epilepsy, cerebellar dysfunction, peripheral nerve hyperexcitability, insomnia, autonomic dysfunction, neuropathic pain or weight loss. This repertoire of symptoms is consistent with the syndromes previously ascribed to this autoantibody in two series including 19 and 8 patients.[4,12]

Two frequent symptoms included neuropathic pain (61%) and cerebellar dysfunction (35%). Pain was previously described in patients with Morvan's syndrome and Caspr2-antibodies[6,15] and it was attributed to small fiber polyneuropathy.[15] Unremarkable nerve conduction studies in 67% of our patients support this hypothesis. Most patients with cerebellar symptoms had other additional clinical features, such as LE.[12,16,17] Our findings suggest that cerebellar symptoms in patients with limbic

encephalitis should raise suspicion of Caspr2-antibodies, although similar clinical features can occur in patients with antibodies to GABAb receptor[18], Hu[19] or in children with NMDAR-antibodies.[20]

In 30% of the patients the disease evolved in more than 1 year, which is in contrast to the subacute onset of most antibody-associated encephalitis.[21] This protracted course of the disease can lead to diagnostic delays or to misdiagnosing the disorder as a primarily neurodegenerative disease, preventing the early use of immunotherapy. The low sensitivity of CSF pleocytosis adds to this difficult distinction. The VGKC-RIA does not always test positive either, so Caspr2 should be specifically requested.

Most patients with confirmed Caspr2-antibodies were male (89%), which is in line with earlier reports (84-88%).[4,12] Autoimmune diseases are generally considered to be more frequent in women, but male predominance is also seen in late onset myasthenia gravis.[22] The reason for this male predominance is unclear. Although the expression of Caspr2 mRNA in the prostate was suggested[6], Caspr2 mRNA is also expressed in the ovaries[23]. Interestingly, the few women of our study were younger than men, frequently had an underlying tumor (none of them of the ovary), and showed high propensity to autoimmunity (MG and LGI1-antibodies).

Twenty-five percent of the patients had relapses, some of which occurring up to 7 years after the initial episode of the disease. Considering that the overall median follow-up was 3 years, the late relapses of some patients may suggest an even higher relapse rate. In almost half of the cases with relapses the initial diagnosis of the disease was made during the relapse, suggesting that patients with a monophasic disease may be missed at disease onset leading to an overestimation of relapse rates. This occurred in other autoimmune encephalitis such as anti-AMPAR[24] and anti-NMDAR in which a drop of relapse rate was noted after these disorders were better recognized and promptly diagnosed.[20] Similar to these encephalitides, about half of the relapsing cases with Caspr2-antibodies were not appropriately treated in the first episode. The lower relapse rate in treated patients versus untreated patients is similar to that seen in anti-NMDAR encephalitis.[20] One should be aware that in patients with Caspr2-antibodies the symptoms at relapse may involve different parts of the nervous system than those involved in the initial episode (e.g. CNS or peripheral nervous system).

A limited number of autoimmune disorders are associated with IgG4 antibodies. Recently, IgG4 antibodies were demonstrated in several anti-LGI1 patients and in three out of seven anti-Caspr2 patients.[6] All our patients had IgG4 antibodies against Caspr2; this finding is important for a better understanding of the pathophysiology of the disease and has treatment implications. IgG4 antibodies have the property of being functionally monovalent through the in vivo exchange of IgG half-molecules (one H- plus one L-chain).[25] Therefore, in contrast to IgG1 antibodies (as in anti-NMDAR encephalitis) IgG4 antibodies are unable to crosslink the target leading to its

internalization. Moreover, IgG4 antibodies show low affinity for the Fcγ receptor, and are inadequate in activating cellular immune responses and complement. We postulate that IgG4 Caspr2-antibodies may be directly pathogenic by altering Caspr2-related cell-to-cell interactions.

A limitation of this study is the retrospective collection of data obtained from medical records. Therefore, we possibly overestimated the true frequency of some symptoms, as missing information was not taken into account. Nevertheless, the current findings will improve the recognition of the core symptoms associated to Caspr2-antibodies as well as the frequent protracted clinical course and high relapse rate. Early recognition is important because our data confirm that immunotherapy and tumor treatment (if needed) are often effective in this disease.

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Supplemental material

Laboratory Studies

Cell based assay (CBA) was performed with an in-house test at Hospital Clinic, whereas the Erasmus Medical Center used a commercially available test (Euroimmun, Lübeck, Germany). For both tests, fixed human embryonic kidney 293 cells had been transfected with cDNA encoding Caspr2 protein. The in-house test was performed with serum diluted 1:40 and CSF 1:5, and Alexa Fluor 488 goat anti-human IgG (1:1000; Invitrogen) to detect bound IgG. Dilutions for the commercial CBA were serum 1:10 and CSF undiluted, with fluorescein isothicyanate (FITC)-conjugated goat-anti-human IgG to detect bound IgG, according to manufacturer's instructions. Serum IgG subtyping was performed using CBA and FITC conjugated sheep anti–human IgG1 (1:400) or IgG4 (1:1000) as secondary antibodies (The Binding Site, Birmingham, England). All assays were examined with a fluorescence microscope by two of the investigators.

IHC was performed according to similar protocols in both laboratories. In brief, paraformaldehyde fixed rat brain was prepared as previously reported[1], and 7 μm thick sagittal sections were serially incubated with 0,3% H2O2 for 15 minutes, 5% goat serum for 60 minutes, and overnight with patient's serum (1:200) or CSF (1:2) at 4˚C. Subsequently, sections were incubated with biotinylated goat anti-human IgG, avidin-biotin peroxidase and the reactivity developed with diaminobenzidine. IHC was considered positive if a previously reported neuropil staining pattern characteristic of Caspr2 antibodies was identified.[2]

Sera were tested for VGKC-complex antibodies with radioimmunoassay (RIA) in the Erasmus Medical Center, using 125I-α-dendrotoxin labeled VGKC extracts of mammalian brain, according to the manufacturer's instructions (DLD Diagnostika GmbH, Hamburg, Germany). Results were expressed as picomoles of 125I-α-dendrotoxin binding sites precipitated per liter of serum, corrected for mean results of control samples. Samples < 50 pM were considered negative. All samples > 50 pM were tested twice. Results ranging from 50 to 100 pM were inconclusive, results > 100 pM were considered positive.

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Table e-1: Caspr2 core symptoms in 38 patients with confirmed Caspr2 antibodies

	Gender	Age	Subacute onset	No. symptoms present	No. symptoms documented	CNS	Cerebellar	PNH	Autonomic	Insomnia	Pain	Weight loss
1	M	69	No	6	7							
2	F	53	Yes	6	7							
3	M	77	No	6	7							
4	M	74	No	6	7							
5	F	45	No	6	7							
6	F	45	No	5	5							
7	M	68	No	5	6							
8	M	71	Yes	5	6							
9	M	62	Yes	5	7							
10	M	69	No	5	7							
11	M	74	No	5	7							
12	M	54	Yes	5	7							
13	M	68	No	4	5							
14	M	68	No	4	7							
15	M	66	No	4	7							
16	M	62	No	4	7							
17	M	61	No	4	7							
18	M	53	Yes	4	7							
19	M	74	Yes	4	7							
20	M	61	No	3	4							
21	M	57	Yes	3	4							
22	M	59	No	3	7							
23	M	62	Yes	3	7							
24	M	76	No	3	7							
25	M	66	No	3	7							
26	M	40	No	3	7							
27	M	74	No	2	3							
28	M	25	Yes	2	3							
29	M	72	Yes	2	5							
30	M	60	Yes	2	7							
31	M	72	N.A.	1	7							
32	M	68	Yes	2	7							
33	M	75	Yes	1	6							
34	M	74	No	2	7							
35	M	62	No	1	6							
36	F	58	Yes	1	4							
37	M	67	N.A.	1	4							
38	M	58	No	1	3							

Subacute onset = progression to maximum disease severity in three months
Green = symptom present. **Orange** = symptom absent. **Grey** = symptom not documented.

Table e-2: Symptoms and diagnosis in 10 patients with antibodies detected only with CBA (without confirmation with brain IHC)

	M/F	Age	Subacute onset	No. symptoms present	No. symptoms documented	CNS	Cerebellar	PNH	Autonomic	Insomnia	Pain	Weight loss	Final diagnosis or clinical description
1	M	83	No	4	7								Creutzfeldt Jakob disease
2	F	28	No	3	7								Cramp fasciculation syndrome
3	M	56	Yes	2	2								Autoimmune encephalopathy
4	M	63	No	2	6								Encephalopathy with hypokinetic rigidity
5	M	78	No	2	7								Creutzfeldt Jakob disease
6	F	62	Yes	1	2								Psychiatric symptoms
7	F	37	Yes	1	2								Neuro-SLE
8	F	83	Yes	1	2								Episodic cognitive disturbance
9	M	62	No	1	7								Limbic encephalitis
10	M	78	No	1	7								Limbic encephalitis

Subacute onset = progression to maximum disease severity in three months
Green = symptom present. **Orange** = symptom absent. **Grey** = symptom not documented.

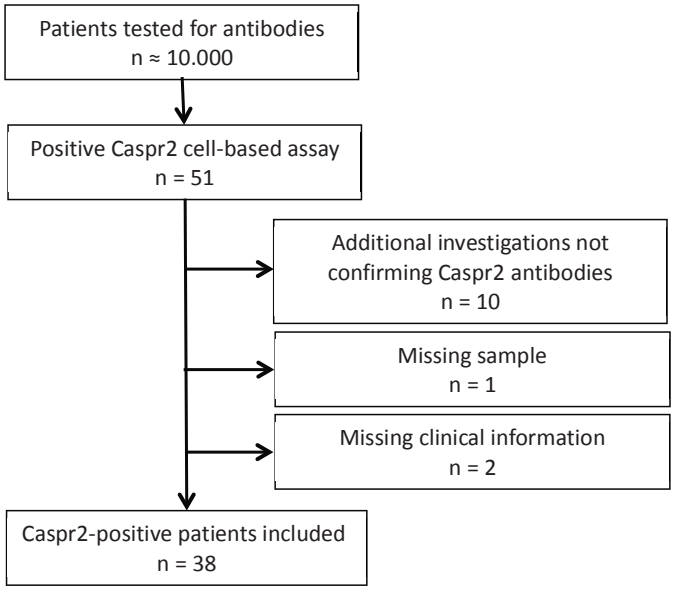
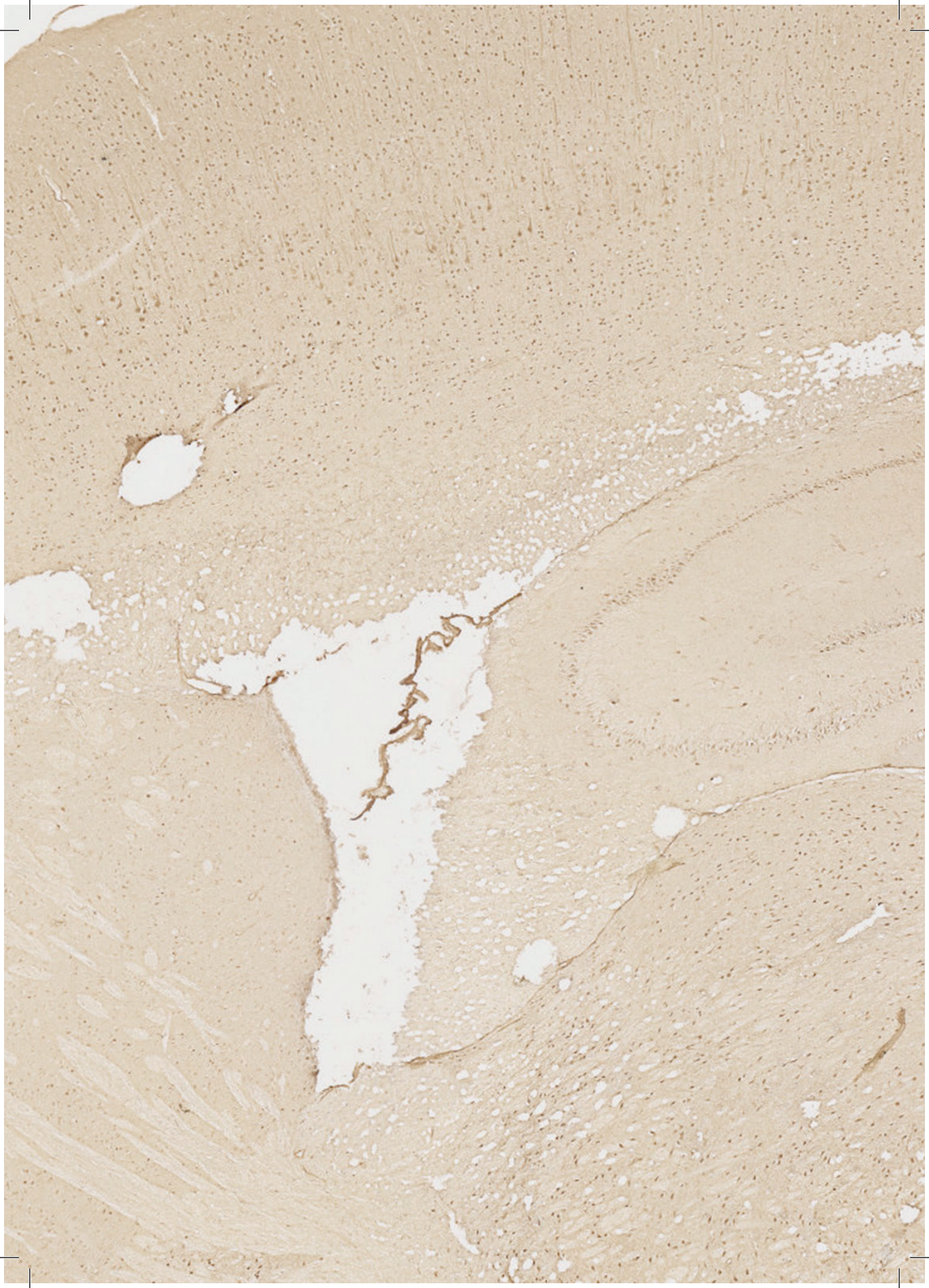


Figure e-1: Flow chart showing inclusion for the study



CHAPTER 5

The relevance of VGKC-positivity in the absence of LGI1 and Caspr2 antibodies

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Abstract

Objectives

This study assessed the clinical relevance of a positive voltage-gated potassium channel (VGKC)-test in patients lacking antibodies to LGI1 and Caspr2.

Methods

VGKC-positive patients were tested for LGI1 and Caspr2 antibodies. Patients lacking both antibodies were matched (1:2) to VGKC-negative patients. Clinical and paraclinical criteria were used to blindly determine evidence for autoimmune inflammation in both groups. Patients with an inconclusive VGKC-titer were analyzed in the same way.

Results

1455 patients were tested by VGKC radioimmunoassay. 56 patients tested positive, of which 50 patients were available to include. 25 patients had antibodies to LGI1 (n=19) or Caspr2 (n=6) and 25 patients lacked both antibodies. Evidence for autoimmune inflammation was present in 7 (28%) of the VGKC-positive patients lacking LGI1 and Caspr2, compared to 9 (18%) of the VGKC-negative controls ($p=0.38$). Evidence for autoimmune inflammation was mainly found in patients with limbic encephalitis/encephalomyelitis (57%), but not in other clinical phenotypes (5%, $p<0.01$). VGKC-titers were significantly higher in patients with antibodies to LGI1 or Caspr2 ($p<0.001$). However, antibodies to Caspr2 could also be detected in patients with inconclusive low VGKC-titer while many VGKC-positive patients had no evidence for autoimmune inflammation

Conclusions

VGKC-positivity in the absence of antibodies to LGI1 and Caspr2 is not a clear marker for autoimmune inflammation and seems not to contribute in clinical practice. No cut-off value for the VGKC-titer was appropriate to discriminate between patients with and without autoimmune inflammation.

Introduction

Voltage-gated potassium-channel complex antibodies (VGKC) were initially detected in patients with neuromyotonia, Morvan's syndrome and limbic encephalitis (LE).[1-3] Samples were positive in the VGKC-radioimmunoassay but did not show reactivity to cells transfected with VGKCs. Major progression was made in 2010 with the detection of antibodies to proteins associated with the VGKC-complex: leucine-rich glioma-inactivated protein1 (LGI1) and contactin-associated protein-like 2 (Caspr2).[4,5] LGI1-antibodies are associated with limbic encephalitis, while Caspr2-antibodies can cause both central and peripheral nervous system symptoms.[4,6]

A substantial part of the VGKC-positive patients lack antibodies to both LGI1 and Caspr2. In literature these 'double negative' patients are often grouped together with LGI1/Caspr2-positive patients, although it is unknown if pathogenic mechanisms are similar. At least clinically, these 'double negative' patients form an essentially distinct subgroup. Increasing numbers of patients are published with a myriad of symptoms, including epilepsy,[7] neuropathic pain,[8] and even Creutzfeldt-Jakob disease (CJD).[9] While the clinical spectrum of 'double negative' patients is broadening, it is unclear if VGKC-positivity in these patients reflects clinical relevance.[10] At least in some patients, i.e. with CJD, autoimmune etiology is proven unlikely, but this has not been studied on group level. It is also unclear whether VGKC-titer levels adequately assess clinical relevance.We assessed the probability of an autoimmune basis for disease in VGKC-positive patients without LGI1/Caspr2 antibodies, compared to matched VGKC-negative patients. The clinical relevance of VGKC-titer levels is explored. The aim of this study is to assess the clinical relevance of a positive VGKC-test in patients without antibodies to LGI1/Caspr2.

Methods

Patients and clinical evaluation

The Erasmus University Medical Center is the national referral center for neuronal and extracellular antibody testing in the Netherlands. Serum samples had been sent for radioimmunoassay (RIA) for VGKC-complex antibodies between January 2008 and December 2013. Patients with a positive or inconclusive VGKC-RIA were tested for LGI1 and Caspr2 antibodies, as well as matched negative controls. VGKC-positive patients lacking both antibodies were the main study group. The patients with an inconclusive VGKC-RIA were analyzed separately.

VGKC-positive patients without antibodies to LGI1/Caspr2 were matched with VGKC-negative patients. Matching (1:2) was based on clinical syndrome (as noted in free text by the referring physician), age (±5 years) and gender. The first two matching patients from a consecutive list of VGKC-negative test results were selected. Clinical

information from patients and controls was obtained retrospectively from the treating physician and additional laboratory tests were performed as described below. Treatment response was only included in the analysis if explicitly noted in the patient file. Two investigators (MT/PSS) were blinded for VGKC-test result and (effect of) immunotherapy, and independently reviewed evidence for autoimmune inflammation in all cases. Evidence for autoimmune inflammation was based on rigorous clinical criteria and supporting results of ancillary testing, Table 1. These criteria were established upfront of the current study, since there is no consensus on international diagnostic criteria for autoimmune encephalitis yet. The investigators assessed each criterion (A-D) separately and finally concluded whether the patient met the criteria for autoimmune inflammation. If in disagreement, reviewers convened to achieve agreement. Additionally, the investigators rated the probability of autoimmune basis for disease for each patient on a 0-10 point scale. Higher scores indicate a higher likelihood of autoimmune etiology. The mean of the scores of the two reviewers was calculated as 'autoimmune rating score'. Investigators also defined the clinical subtype.

Table 1. Criteria for autoimmune classification.

Evidence for autoimmune inflammation when fulfilling criteria A, B, C1 or C2, and D
A. Subacute onset of disease (substantial part of disease within 3 months)
B. Clearly defined clinical syndrome*: - Limbic encephalitis - Morvan's syndrome - Encephalomyelitis / progressive encephalomyelitis with rigidity and myoclonus (PERM) - Brainstem encephalitis - New onset focal epilepsy - Psychosis - Neuromyotonia
C1. Antibody detected: NMDAR, AMPAR, DPPX, GABAaR, GABAbR, GAD65, aquaporin-4 antibodies or classical onconeural antibodies** Antibodies detected in both serum and CSF, or confirmation with immunohistochemistry or neuronal cell culture (not needed in aquaporin-4 antibodies) or **C2: ≥ 2 results of ancillary tests supporting autoimmune diagnosis:** - CSF: pleiocytosis and/or unmatched oligoclonal bands - Brain MRI: abnormalities consistent with autoimmune inflammation, i.a.T2 hyperintens temporal lobe lesion - Tumor: systemic tumor present within two years of neurological diagnosis - Positive serum immunohistochemistry and neuronal cell culture, or positive CSF immunohistochemistry or neuronal cell culture - Serological markers for autoimmune disease, i.a. anti-TPO - Histopathological evidence for autoimmune inflammation (brain biopsy or autopsy)
D. No other diagnosis

*according to Graus et al[19] Syndromes irrelevant for the current study are omitted. Morvan's syndrome, new onset focal epilepsy and psychosis were added according to recent literature [4,20,21]

**antibodies to Hu, Ri, Yo, Tr, CV2, Ma1, Ma2, amphiphysin

Patients and controls were classified in four subgroups. The first and second group consisted of patients with antibodies to LGI1 or Caspr2, respectively. The third group consisted of patients without antibodies to LGI1/Caspr2 but fulfilling criteria for autoimmune inflammation ('autoimmune' group). The fourth group were patients not meeting these criteria ('non-autoimmune' group)

Laboratory investigation

All assays have been performed as described before. Methods are described in short below, and in more detail in the Supplemental material.

Sera were tested for antibodies to the VGKC-complex by commercial RIA, using 125I-α-dendrotoxin-labeled VGKC-extracts of mammalian brain, according to the manufacturer's instructions (DLD Diagnostika GmbH, Hamburg, Germany). Results were expressed as picomoles (pM) of toxin bound per liter of test serum, corrected for mean results of control samples. Samples <50 pM were considered negative, results ranging from 50 to 100 pM inconclusive and titers >100 pM were considered positive. These cut-off values are based on extensive validation studies with an external laboratory. Patients were tested for LGI1 and Caspr2 antibodies using commercial cell-based immunofluorescence assay (CBA) of fixed human embryonic kidney293 cells transfected with cDNA encoding the relevant protein (Euroimmun, Lübeck, Germany). Both positive and negative samples were additionally screened for antibodies to cell-surface antigens with immunohistochemistry on rat brain. This screening test can show antigen-specific staining patterns, i.e. NMDAR and LGI1, or nonspecific diffuse neuropil staining, i.e. Caspr2, AMPAR and GABAbR. When results were contradictory, samples were tested on live hippocampal neuronal cell cultures for confirmation. Tests were performed on serum samples, and CSF if available.

Immunoblot assays were used to test for antibodies to classical onconeural antigens (Hu/Ri/Yo/Tr/CV2/Ma1/Ma2/amphiphysin) (Ravo Diagnostika GmbH, Freiburg, Germany and Euroimmun, Lübeck, Germany) and CBAs were used to test for other extracellular antibodies, including antibodies directed to NMDAR/GABAbR /AMPAR (Euroimmun, Lübeck, Germany, performed according to manufacturer's instructions), or DPPX/GABaR/AQP4/GlycineR (in house assay).

Standard protocol approvals, registrations and patient consents

The Institutional Review Board of the Erasmus University Medical Centre approved the study. Informed consent was obtained in all patients.

Statistical analysis

Inter-rater agreement for the criteria of autoimmune inflammation was analyzed with Cohen's kappa for categorical data. Intra-class correlation coefficient was used to assess inter-rater agreement of the 0-10 point rating scale. Subgroups were compared with

Fisher-Freeman-Halton exact test for categorical data. Numerical data were analyzed with Mann Whitney-U test (2 groups) or Kruskal-Wallis test (>2 groups). The relation between mRS or delay of testing with VGKC-RIA titer was tested with Spearman's rank correlation coefficient. Statistical significance was defined as p-value <0.05, with Bonferroni correction for multiple testing in post hoc analysis. SPSS Statistics 21 (IBM Corp., Armonk, NY) was used for analysis.

Results

1455 patients had been tested by VGKC-RIA, of which 51.5% were male and 8.6% were children under age 18. 56 patients (3.8%) had a positive result (titer>100pM) and 41 patients (2.8%) had an inconclusive test result (titer 50-100pM).

50 VGKC-positive patients were included in the study, including three children. Six patients were excluded because data were missing. Median age at onset was 62 years (range 1–84 years). 54% of the patients were male. 19 patients had antibodies to LGI1 and six patients were positive for Caspr2 antibodies. 25/50 patients (50%) were negative for antibodies to LGI1/Caspr2 ('double negatives') and were matched to 50 VGKC-negative patients. 32 patients with an inconclusive VGKC-result were analyzed, of which 30 patients were negative for LGI1/Caspr2 antibodies. See Figure 1

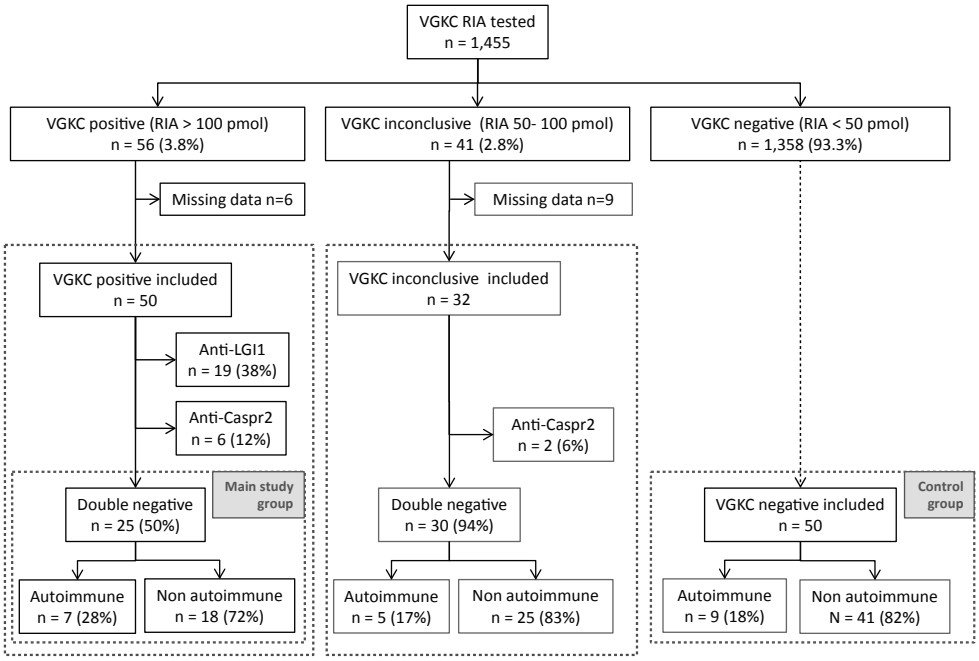

Figure 1: Flow chart for the inclusion of patients and the comparison of subgroups. Serum samples from 1,455 patients had been tested by anti-VGKC radioimmunoassay. Dashed red blocks: 25 VGKC-positive patients lacking antibodies to LGI1 and Caspr2 (main study group) were compared to 50 matched VGKC-negative patients (control group). Dashed blue blocks: titer analysis included 50 VGKC-positive and 32 VGKC-inconclusive patients.

Interrater agreement

105 LGI1/Caspr2-antibody negative cases were reviewed for evidence for autoimmune inflammation by two investigators independently (25 VGKC-positive, 50 VGKC-negative and 30 VGKC-inconclusive patients). Cohen's kappa for final conclusion (autoimmune or non-autoimmune) was κ=0.94 (Supplemental material). The probability for autoimmune disease, as scored on a 0-10 point rating scale, had an intra-class correlation coefficient of 0.88 (95%CI 0.83–0.92). The median difference between the reviewers' scores was 0 (range -4 to +3), and only in 4 patient rating scores differed >2 points.

VGKC-positive cohort

50 VGKC-positive patients were analyzed. The clinical and paraclinical features varied between the four subgroups (Table 2). The LGI1 and Caspr2 patients are not discussed further. The double negative patients with and without evidence for autoimmune inflammation are described below, and subsequently compared to matched VGKC-negative patients.

7/25 (28%) VGKC-positive patients without LGI1/Caspr2 antibodies fulfilled criteria for autoimmune inflammation. The clinical picture consisted of LE (n=4, one patient had additional ataxia) or encephalomyelitis (EM) (n=3). Another antibody was detected in two patients: anti-NMDAR and anti-Aquaporin4 antibodies respectively. In the other five patients, evidence for auto-immune inflammation was based on supporting test results.

18/25 (72%) VGKC-positive patients lacking LGI1/Caspr2 antibodies had insufficient clinical and paraclinical evidence for autoimmune inflammation. Clinical phenotypes were more heterogeneous, including LE, epilepsy, psychiatric symptoms, cramp fasciculation syndrome, myoclonus and polyradiculopathy. Patients did not fulfil criteria for subacute onset (n=4), had less than two results of ancillary tests supporting autoimmune inflammation (n=18) or another diagnosis was established (n=2, leptomengeal metastasis, intracerebral bleeding). All three VGKC-positive children lacked evidence for autoimmune inflammation.

Table 2. VGKC-positive patients classified in subgroups (n = 50)

	Anti-LGI1 (n = 19, 38%)	Anti-Caspr2 (n = 6, 12%)	Autoimmune (n = 7, 14%)	Non autoimmune (n = 18, 36%)	p-value
Median age at onset (range)	61 (31-84)	73 (62-83)	50 (18-75)	58,5 (1-79)	0.030*
Male gender	10/19 (53%)	6/6 (100%)	4/7 (57%)	7/18 (39%)	0.069
Clinical subtype					
- Limbic encephalitis	16 (84%)	4 (67%)	4 (57%)	3 (17%)	<0.001*
- Morvan's syndrome	2 (11%)	0	0	0	0.72
- Encephalomyelitis/PERM	0	0	3 (43%)	0	0.003*
- Brainstem encephalitis	0	1 (17%)	0	0	0.12
- New onset focal epilepsy	1 (5%)	1 (17%)	0	1 (6%)	0.57
- Psychosis	0	0	0	2 (11%)	0.58
- Neuromyotonia	0	0	0	1 (6%)	0.62
- Other	0	0	0	11 (61%)	<0.001*
Cognitive symptoms	18/19 (95%)	5/6 (83%)	6/7 (86%)	12/18 (67%)	0.13
Epilepsy	17/19 (89%)	5/6 (83%)	4/7 (57%)	7/18 (39%)	0.006*
FBDS	10/19 (53%)	0/6 (0%)	0/7 (0%)	0/18 (0%)	<0.001*
PNS symptoms	2/19 (11%)	2/6 (33%)	0/6 (0%)	3/18 (17%)	0.35
Sleep disorder	5/10 (50%)	1/3 (33%)	0/2 (0%)	4/10 (40%)	0.81
Hyponatremia (serum sodium < 135 mmol/L)	12/18 (67%)	1/6 (17%)	0/7 (0%)	3/16 (19%)	0.004*
CSF					
- Cell count > 5 cell/µL	1/18 (6%)	1/5 (20%)	4/7 (57%)	2/13 (15%)	0.030*
- Protein > 0.58 g/L	2/18 (11%)	1/5 (20%)	3/6 (50%)	0/13 (0%)	0.029*
MRI					
- Abnormalities limbic area	7/19 (37%)	2/6 (33%)	2/6 (33%)	1/13 (8%)	0.24
- Other abnormalities	0/19 (0%)	1/6 (17%)	2/6 (33%)	8/13 (62%)	<0.001*
- Normal	12/19 (63%)	3/6 (50%)	2/6 (33%)	4/13 (31%)	0.31
EEG					
- Epileptic	8/18 (44%)	3/5 (60%)	2/6 (33%)	5/11 (45%)	0.91
- Slow	8/18 (44%)	0/5 (0%)	5/6 (83%)	7/11 (64%)	0.033*
- Normal	5/18 (28%)	2/5 (40%)	0/6 (0%)	1/11 (9%)	0.24
Tumor	2/19 (11%)	0/6 (0%)	1/5 (20%)	4/13 (31%)	0.27
VGKC RIA titer, median (range)	936 (245 – 1314)	412 (385 – 653)	148 (105 – 295)	117 (102-219)	<0.001*

*p < 0.05 (Fisher-Freeman-Halton exact test, comparing the four groups).
PERM = progressive encephalomyelitis with rigidity and myoclonus. FBDS = faciobrachial dystonic seizures. PNS = peripheral nervous system. CSF = cerebrospinal fluid. MRI = magnetic resonance imaging. EEG = electroencephalography. RIA = radioimmunoassay.

VGKC-positive vs VGKC-negative patients

The VGKC-positive patients without LGI1/Caspr2 antibodies (n=25) were compared to matched VGKC-negative patients (n=50), Table 3. None of the criteria for autoimmune inflammation differed. There was no difference in the proportion of patients with evidence for autoimmune inflammation in the VGKC-positive 'double negative' group compared to VGKC-negative patients (28% vs 18%, p=0.38).

Table 3. VGKC-positive (LGI1/Caspr2 negative) vs VGKC-negative patients

	VGKC-positive LGI1/Caspr2 negative n = 25	VGKC-negative n = 50	
Subacute onset	21/25 (84%)	39/50 (78%)	p = 0.76
Ancillary tests supporting autoimmune diagnosis			
Antibody detected	2/25 (8%)[a]	3/50 (6%)[b]	p = 1.00
Brain MRI	7/19 (37%)	12/41 (29%)	p = 0.57
CSF	7/20 (35%)	14/41(34%)	p = 1.00
Tumor detected	5/18 (28%)	6/28 (21%)	p = 0.73
Immunohistochemistry and cell culture	4/25 (16%)	6/50 (12%)	p = 0.72
Serological markers	1/12 (8%)	6/29 (21%)	p = 0.65
Histopathology	2/2 (100%)	1/3 (33%)	p = 0.40
Other diagnosis	2/25 (8%)	9/50 (18%)	p = 0.32
Evidence for autoimmune inflammation	7/25 (28%)	9/50 (18%)	p = 0.38
Autoimmune rating score, medianc	3,5	2	p = 0.14

[a]Antibodies detected: anti-NMDAR, -Aquaporin-4

[b]Antibodies detected: anti-NMDAR (2x), -GAD65 serum titer > 50.000 IU/mL, CSF titer > 1000 IU/mL. NMDAR and GAD65 antibodies confirmed with immunohistochemistry.

[c]Probability expressed on a 0-10 point scale. Patients with 'other certain diagnosis' excluded (1 VGKC-positive patient and 9 VGKC-negative patients)

VGKC-inconclusive patients

41/1455 patients (2.8%) had an inconclusive VGKC-RIA (titer 50-100pM). Nine patients were excluded because data were missing. 32 VGKC-RIA inconclusive patients were included in the subgroup analysis. Median age at onset was 62 years (range 2–83 years). 56% of the patients were male. Two patients (6%) had Caspr2-antibodies, presenting with either LE or Morvan's syndrome. LGI1-antibodies were not detected. 30 patients (94%) were double negative, of which 5 patients (17%) met criteria for autoimmune inflammation. One of them had GABAbR-antibodies. There was insufficient evidence for autoimmune inflammation in 25/30 (83%) VGKC-inconclusive patients without LGI1/Caspr2 antibodies. Among them were a patient with glioblastoma multiforme, a patient with progressive multifocal leukoencephalopathy and a patient with histopathologically proven CJD.

Therapy and outcome

Clinical outcome was known in 111 of the 132 patients (84%). 17/19 (89%) of the anti-LGI1 patients and 8/8 (100%) of the anti-Caspr2 patients showed substantial clinical improvement. Of patients lacking LGI1/Caspr2 antibodies, improvement was noted in 11/24 (46%) of the VGKC-positive patients, 20/29 (69%) of the VGKC-inconclusive patients and 23/31 (74%) of the VGKC-negative patients (p=0.087).

In patients without antibodies to LGI1/Caspr2 improvement on immunotherapy was noted in 6/12 (50%) VGKC-positive, 7/13 (54%) VGKC-inconclusive and 9/11 (82%) VGKC-negative patients (p=0.25). In these patients, those with evidence for autoimmune inflammation showed more effect of immunotherapy (12/14 [86%]) than

the non-autoimmune group (10/22 [45%], p=0.033). Improvement on therapy was seen in 15/15 (100%) of the anti-LGI1 patients and 6/7 (86%) of the anti-Caspr2 patients, irrespective of VGKC-results. Five patients were excluded for analysis of therapeutic response because they had disease known to improve on immunotherapy, but unrelated to antibodies (Guillain-Barre syndrome[3], chronic inflammatory demyelinating polyneuropathy[1], Behçet's disease[1]).

Relevance of VGKC-titer

VGKC-titers differed between the four subgroups (p<0.001; Figure 2). However, the subgroups showed considerable overlap of VGKC-titers. The shortcoming of current cut-off values is depicted by the two patients with Caspr2 antibodies while VGKC-RIA results were inconclusive, and by the high amount of patients with a positive VGKC-result without evidence for autoimmune inflammation. However, no change of cut-off values would obviate both problems.

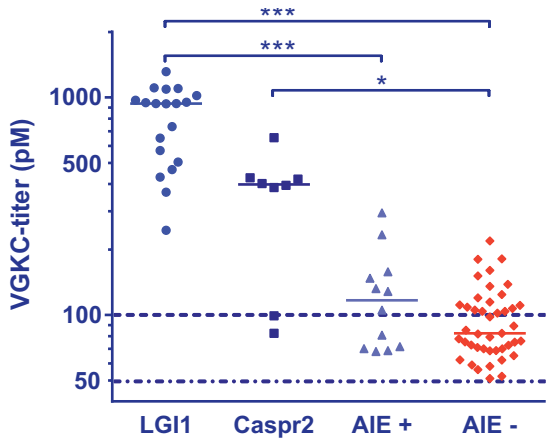

Figure 2: Anti-VGKC RIA positive and inconclusive titers. Titer levels differ significantly between subgroups (p<0.001). *p < 0.05; *** p < 0.0005; AIE+ = subgroup of patients with evidence for autoimmune inflammation; AIE- = subgroup op patients without evidence for autoimmune inflammation.

VGKC-titer was not correlated with disease severity measured with mRS. There was no correlation between delay from onset of disease to VGKC testing and VGKC-titers (data not shown).

Relevance of clinical syndrome

Probability for autoimmune inflammation was independent of VGKC-result, but strongly associated with clinical syndrome. Clinical syndrome was defined by the independent investigators for all patients without LGI1/Caspr2 antibodies (n=105). 57% of the patients with LE or EM had evidence for autoimmune disease compared to 5% of the patients with other clinical syndromes (p<0.01). (Suppl. Figure e-1)

Discussion

This study investigated the clinical relevance of VGKC-positivity in patients without LGI1/Caspr2 antibodies by analyzing evidence for autoimmune inflammation, compared to matched VGKC-negative patients. We provide some relevant findings: 1) Half of the VGKC-positive patients lack antibodies to LGI1 and Caspr2, 2) There is no evidence that VGKC-positivity in the absence of antibodies to LGI1/Caspr2 is a marker for autoimmune inflammation, 3) No cut-off value for the VGKC-titer is appropriate to discriminate between patients with and without autoimmune inflammation.

Half of the VGKC-positive patients had antibodies to LGI1 (38%) or Caspr2 (12%). Other studies report LGI1 or Caspr2 antibodies in 26–77% of the VGKC-positive patients. The higher percentages are reported in cohorts of patients with higher VGKC-RIA titer cut-offs and in studies only including patients with LE.[4,11-13] This relation with LE also applies to our cohort (77% of LE patient had LGI1 or Caspr2 antibodies).

We compared VGKC-positive patients without LGI1/Caspr2 antibodies to matched VGKC-negative patients. This comparison did not reveal differences between these two groups on any of the assessed criteria. Evidence for autoimmune inflammation was present in 28% of VGKC-positive patients without LGI1/Caspr2 antibodies and 18% of VGKC-negative patients. Although we cannot exclude that some of these patients with LE or EM have an autoimmune disease somehow related to the VGKC-complex, this small non-significant difference is most likely due to imperfect matching. Matching was based on the clinical syndrome noted on the request form, which in some cases did not correspond with the final diagnosis defined by the investigators. Five VGKC-positive patients with LE were matched to a VGKC-negative patient with less-defined CNS symptoms, and in one the mismatch was in the opposite way. This resulted in a lower incidence of LE/EM in the control group (28% vs 40%), whereas these syndromes were associated with higher incidence of autoimmune inflammation than other syndromes (57% vs 5%). This underlines that matching by clinical syndrome is essential to determine the relevance of diagnostic tests.

VGKC-RIA titers are higher in patients with antibodies to LGI1. This is in line with earlier reports.[12,14] However, we have shown that there is no cut-off value appropriate to discriminate between subgroups. 2/8 (25%) of the anti-Caspr2 patients had an inconclusive VGKC titer (50-100pM) and would have been missed with current cut-off values for positivity. Several anti-LGI1 or anti-Caspr2 patients without VGKC-positivity have been reported before.[6,15] A third of the VGKC-positive patients lacked evidence for autoimmune inflammation, and decreasing cut-off values would further decrease specificity.

Several other studies examined the relevance of the VGKC-test and titer level. High VGKC-titers were associated with CNS symptoms, but these studies included anti-LGI1 patients in titer analysis.[11,14] As we have shown, anti-LGI1 encephalitis is

related to both CNS symptoms and higher VGKC-titers. Previous studies report high tumor rates (10-15%) and response to immunotherapy in >80% of the VGKC-positive patients.[11,12,14] This is in line with our results and might at first sight suggest a paraneoplastic, immune mediated disease. However, both features are seen in matched VGKC-negative patients as well, and are probably more related to clues to request a VGKC-test than to the VGKC-test result itself. The improvement after immunotherapy might reflect true therapeutic response, but could also be natural history, regression to the mean or a secondary immune response, irrespective of the VGKC-result.

On group level, we did not detect relevance of the positive VGKC-test in patients without LGI1/Caspr2 antibodies. The presence of a shared, novel antibody is unlikely in this heterogeneous group of patients, but could be present in a small subgroup of LE or EM patients. Although the presence of a novel antibody should be considered in those patients, it will not apply to the majority of the VGKC-positive patients. The search for (novel) antibodies should be pursued in all patients with high clinical suspicion, and should not be restricted to VGKC-positive patients.

Unlike VGKC-positivity in a substantial part of the patients, most other neurological antibodies are clearly associated with disease. These pathogenic antibodies are directed to (subunits of) a single receptor or protein, such as the NMDA receptor or GABAb receptor. The VGKC-complex is composed of an array of proteins, probably both intracellular and extracellular, and related to neurons and other cell types. Recently, VGKC-positivity was considered a nonspecific marker for neuronal inflammation,[16] but this association remains controversial. VGKC-complex antibodies possibly arise as an epiphenomenon occurring with any kind of cell damage without necessity to be pathogenic or even inflammation-related. This would explain the presence of VGKC-complex antibodies in some patients with prion disease, which we recently detected in another patient and has also been reported by others.[17,18]

The study has some limitations. First, sample size was relatively small, especially after subgroup classification. Contactin-2 antibodies were not tested as these have only been reported in one study without confirmation.[4] Patients were classified according to the evidence for autoimmune disease, after blinding the investigators for VGKC-test result and therapeutic outcome. However, there is no consensus on international diagnostic criteria for autoimmune encephalitis yet and the criteria we used were not verified on other autoimmune disease. Criteria were strict, in order to prevent over diagnosing of autoimmune inflammation. The downside is that we possibly underestimate the number of patients with autoimmune inflammation. This may especially concern patients who do not meet the criteria due to incomplete work up in this retrospective study. However, this affected VGKC-positive patients equally to matched controls, and would not have changed our conclusions. Besides, criteria used are less suitable for auto-immune inflammation restricted to the peripheral nervous system, such as Guillain-Barre syndrome. As 96% of our VGKC-positive patients had

central nervous system symptoms, this limitation will not apply to more than a few patients. Response to immunotherapy was more common in patients fulfilling criteria for autoimmune inflammation, supporting the criteria used. However, outcome was favorable in the majority of the untreated patients as well. This natural course underlines that improvement on immunotherapy does not equal evidence for autoimmune inflammation.

In conclusion, half of the VGKC-positive patients have antibodies to LGI1/Caspr2. These patients usually have high VGKC-titer, but Caspr2-antibodies can be detected in patients with inconclusive, low VGKC-titers as well. No cut-off value for the VGKC-titer is appropriate to discriminate between patients with and without evidence for autoimmune inflammation. When antibodies to LGI1/Caspr2 are lacking, there are no differences between VGKC-positive patients and matched VGKC-negative patients. Clinical reasoning including ancillary testing is leading, whereas VGKC-positivity (by itself) does not contribute in clinical practice.

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Supplemental material

Laboratory methods

Radioimmunoassay (RIA)

In the commercial VGKC autoantibody radioimmunoassay (RIA), performed according to the manufacturer's instructions (DLD Diagnostika GmbH, Freiburg, Germany). VGKC antibody in patient sera and controls (1:10 diluted) are allowed to interact with detergent solubilized VGKCs extracted from rabbit brain tissue and complexed with 125I-labelled α-dendrotoxin (known to react with Kv1.1, 1.2 and 1.6 subtypes of the VGKC). After incubation at 2 – 8 °C overnight, the resulting antigen-antibody complexes are immunoprecipitated by the addition of anti-human IgG. After a second incubation of 1½ hours, assay buffer is added and the samples centrifuged. Unbound 125I-labelled alpha-dendrotoxin-VGKC complex is removed from the tubes by aspiration of the supernatant. The level of radioactivity remaining in the tube is proportional to the antibody. Results were expressed as picomoles (pM) of toxin bound per liter of test serum, corrected for mean results of control samples. Samples < 50 pM were considered negative. All samples > 50 pM were tested twice. Results ranging from 50 to 100 pM were inconclusive, titers > 100 pM were considered positive.

Cell-based immunofluorescence assay (CBA)

LGI1 and Caspr2 antibodies were detected in serum (1:10) or CSF (1:1) using the commercially available Anti-VGKC associated proteins Mosaic 1 cell-based immunofluorescence assay, according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). Fixed human embryonic kidney (HEK) 293 cells transfected with cDNA encoding either LGI1 or Caspr2 protein were incubated with diluted serum (1:10) or CSF (1:2) at room temperature. Unbound antibodies are washed away and bound antibodies are detected by fluorescein isothiocyanate (FITC)-conjugated goat IgG specific for human IgG. Results were evaluated visually by two independent observers using fluorescence microscopy.

Immunohistochemistry

Samples were tested for antibodies to cell-surface antigens with immunohistochemistry on rat brain. Paraformaldehyde (PFA) fixed tissue was prepared as reported before.[1] 7 μm thick sagittal sections were serially incubated with 0,3% H2O2 for 15 minutes, 5% normal goat serum for 30 minutes and overnight with patient's serum (1:200) or CSF (1:2) at 4˚C. Subsequently, sections were incubated with biotinylated goat anti-human IgG (Vector, Peterborough, UK) (1:2000 diluted in 5% normal goat serum) for two hours, avidin-biotin peroxidase (Vectastain Elite ABC complex, Vector Labs,

Burlingame, CA) for one hour and finally with diaminobenzidine (Vector Labs, Burlingame, CA). Slides were dehydrated with ethanol series (50-100%) and mounted with Pertex (Klinipath, Duiven, Netherlands).

Neuronal cell culture

Embryos from pregnant mouse were removed on embryonic days 19 or 20. Hippocampal neurons were prepared as reported before.[2] and grown for 14-17 days. Patient's serum (1:200) or CSF (1:2) was incubated for 1 hour at 37˚C 5% CO2, washed with phosphate-buffered saline (PBS), fixed in 4% PFA for 5 minutes, washed in PBS again and incubated with anti-human IgG (1:1000) for 1 hour in the dark at room temperature.

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Interrater agreement

105 LGI1 and Caspr2 antibody negative cases (25 VGKC-positive, 50 VGKC-negative and 30 VGKC-inconclusive patients) were reviewed by two investigators independently, according to the criteria for autoimmune inflammation (Table 1). For each criterion separately, Cohen's kappa ranged between 0.82 and 1. Cohen's kappa for final conclusion (autoimmune or non-autoimmune) was $\kappa = 0.94$. The investigators drew a different conclusion in two cases (Table e-1). One was a 62-years old female who presented with LE with raised CSF cell count and protein, one year after breast cancer diagnosis. The other patient was a 66 years old male presenting with LE. He had raised CSF protein and anti-TPO antibodies. Differences in evaluation were discussed in a plenary meeting where agreement was reached on classifying both patients in the autoimmune group.

Table e-1. Effect of immunotherapy in VGKC positive, inconclusive and negative patients (number of patients with improvement after treatment / number of patients treated)

	VGKC positive	VGKC inconclusive	VGKC negative	Total
Anti-LGI1	15/15 (100%)	-		-
Anti-Caspr2	4/5 (80%)	2/2 (100%)	-	
Autoimmune	4/6 (67%)	3/3 (100%)	5/5 (100%)	12/14 (86%)
Non autoimmune	2/6 (33%)	4/10 (40%)	4/6 (67%)	10/22 (45%)*

*p = 0.033 (autoimmune vs nonautoimmune)

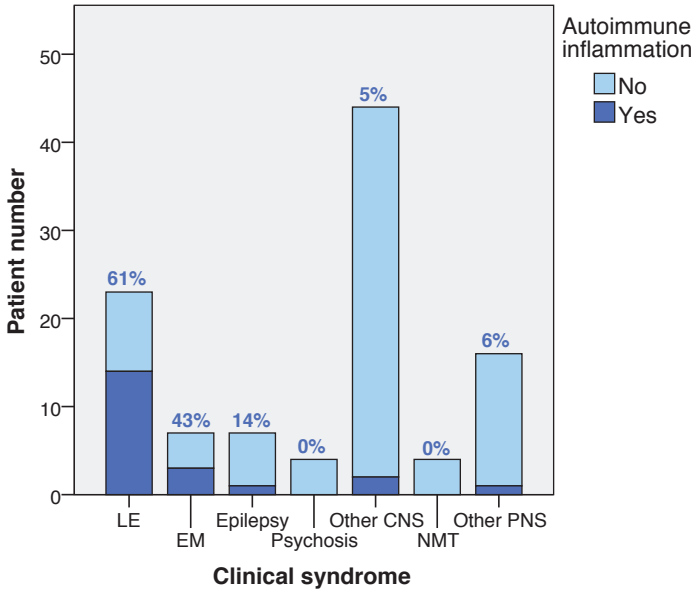

Figure e-1. Evidence for autoimmune inflammation classified according to clinical syndrome.
LE = limbic encephalitis. EM = encephalomyelitis. PERM = progressive encephalomyelitis with rigidity and myoclonus. CNS = central nervous system symptoms. NMT = neuromyotonia. PNS = peripheral nervous system symptoms.

CHAPTER 6

Autoimmune encephalitis and the value of LGI1, Caspr2, and voltage-gated potassium channel (VGKC) antibodies

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Abstract

The discovery of LGI1 and Caspr2 antibodies in 2010 represented a change of views of the clinical significance of voltage-gated potassium channel (VGKC) antibodies. Currently, all these antibodies are still grouped within the term VGKC-complex antibodies, and frequently considered to have a similar clinical value. However, contrary to this concept, recent studies show that the clinical value of these antibodies is different from one another. We review here the clinical significance of these immune responses in 3 settings: patients with antibodies against LGI1, Caspr2, or VGKC-complex but with negative LGI1 and Caspr2 specificity. While the first two antibodies associate with different but well-defined syndromes, the clinical significance of VGKC-complex antibodies without LGI1 and Caspr2 specificity is questionable. We describe each of these syndromes, discuss the function of the target antigens and review the limited pediatric literature on the topic. The findings emphasize the importance of defining these disorders according to the molecular identity of the targets (LGI1, Caspr2), and suggest caution in using VGKC-complex antibodies without further definition of the antigen for the diagnosis and treatment of patients.

Introduction

In the last decade, major progress has been made in the field of antibody mediated neurological diseases. The classical paraneoplastic antibodies, described in the 1980-1990s, are directed to intracellular antigens such as Hu, Ri and Yo. These antibodies associate to neurological syndromes with an inadequate response to immunotherapy and an invariably strong tumor association.[1] In contrast, recently discovered antibodies are directed to extracellular antigens, such as the N-methyl-d-aspartate receptor (NMDAR). These antibodies are thought to be directly pathogenic, and immunotherapy is usually very effective.[2-4] These studies have led to the discovery of over ten novel antibodies against extracellular proteins in the last ten years, including antibodies against leucine-rich glioma-inactivated 1 (LGI1) and contactin-associated protein-like 2 (Caspr2).[5,6] Diseases caused by antibodies to LGI1 or Caspr2 were previously attributed to antibodies against voltage-gated potassium channel (VGKC).

VGKC are present on the membrane of neurons in both the central and peripheral nerve system, where they mediate the repolarization after an action potential. The first report on antibodies directed to the VGKC dates back to 1995, describing plasma exchange responsive patients with acquired neuromyotonia, also known as Isaacs syndrome.[7,8] This peripheral nerve hyperexcitability syndrome is characterized by cramps and impaired muscle relaxation. In the following years, VGKC-antibodies were suspected in patients with Morvan syndrome, showing the combination of neuromyotonia with cognitive symptoms and autonomic dysfunction or insomnia, and in patients with limbic encephalitis.[9,10] Antibodies were detected by radioimmunoassay (RIA), based on 125I-α-dendrotoxin labeled VGKC-complex from mammalian brain. While patients' sera showed positive result on the RIA, all attempts to show reactivity to VGKC-transfected cells failed. This contradiction has led to the discovery that patients in fact did not have antibodies against the VGKC itself, but to VGKC-associated proteins included in the substrate used in the test. (Figure 1 and 2) Two of these proteins were identified in 2010: LGI1 and Caspr2.[5,6]

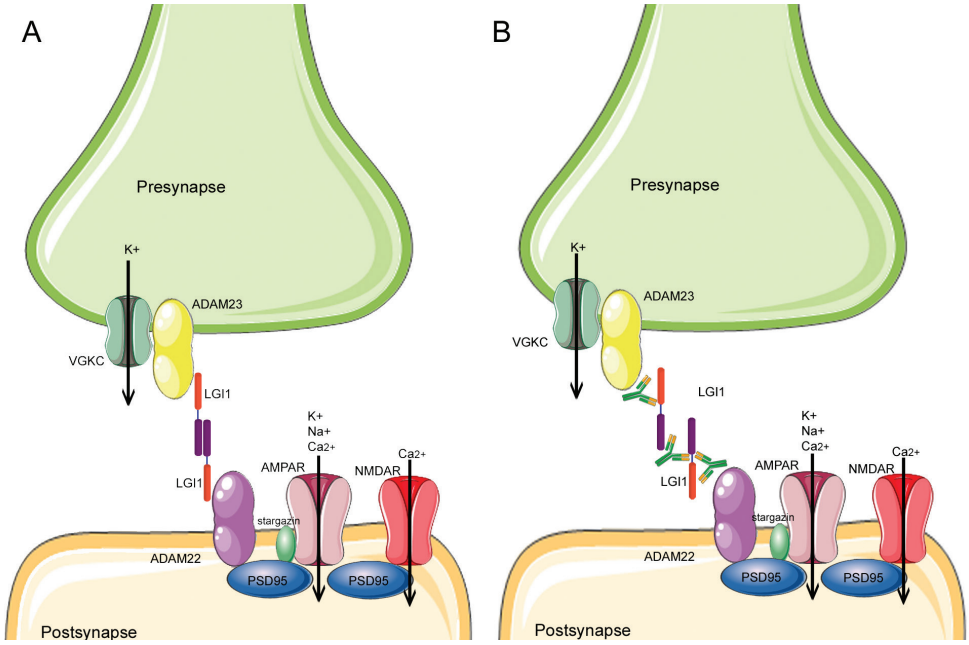

Figure 1. Schematic overview of the LGI1 protein in the synaps. (A) LGI1 dimers bind to presynaptic ADAM23 and postsynaptic ADAM22. This structure regulates AMPAR and VGKC currents. (B) Patients' antibodies bind to LGI1, presumably alterating the neuronal excitability. ADAM = a disintegrin and metalloproteinase. AMPAR =α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. LGI1 = leucine-rich glioma-inactivated 1. NMDAR = N-methyl-d-aspartate receptor. VGKC = voltage-gated potassium channel.

LGI1 is a synaptic protein present in the central nervous system. LGI1 antibodies usually associate with typical limbic encephalitis, seizures and hyponatremia. Caspr2 is present in both the central and peripheral nervous system. This is reflected in the variety of symptoms and presentations associated to Caspr2-antibodies. Both antibodies are more prevalent in older male patients and the syndromes have a common favorable response to immunotherapy. Sera with either LGI1 or Caspr2 antibodies usually show a positive VGKC-RIA result, with higher titer in LGI1-antibody positive patients.[11] It is not uncommon for diagnostic laboratories to start analysis with a VGKC-RIA, followed by cell-based assay to test for LG1 and Caspr2 antibodies after a positive RIA. This two-step approach has two difficulties. First, in a few patients with Caspr2 (or LGI1) antibodies, VGKC-RIA screening is negative, resulting in a missed diagnosis. Second, half of the patients with a positive VGKC-RIA lack antibodies to LGI1 and Caspr2. These patients present with a wide variety of clinical syndromes. The positive VGKC-RIA has prompted many physicians to start immunotherapy. However, recent research has questioned the clinical relevance of a positive VGKC-test in the absence of LGI1 and Caspr2 antibodies.[11]

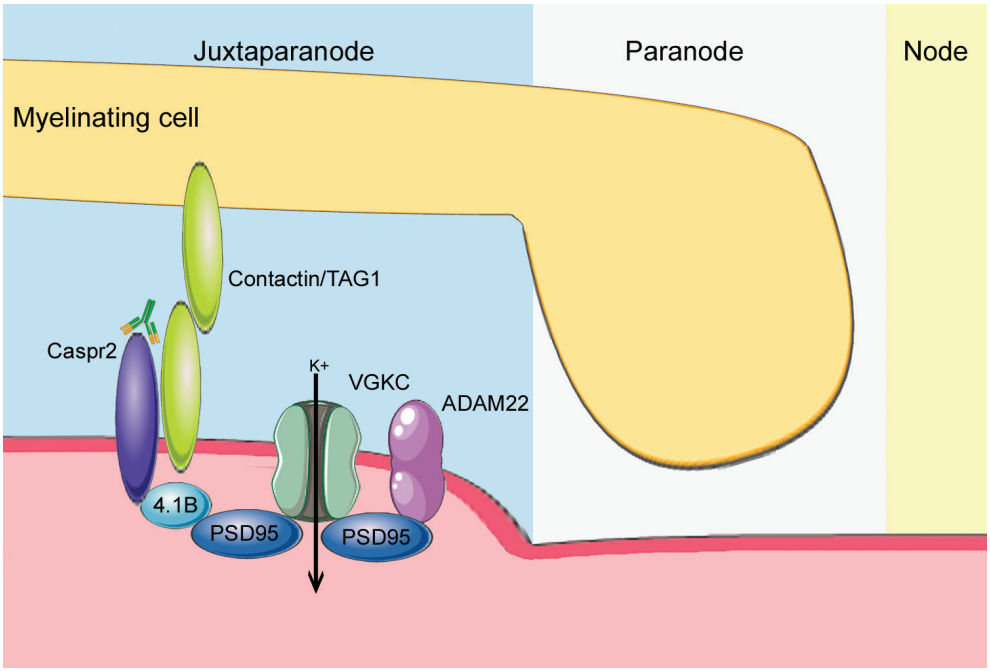

Figure 2. Schematic overview of the Caspr2 protein in the juxtaparanodal region of myelinated axons. Caspr2 connects to the dimerized contactin-2 and to PSD-95. This complex organizes Kv1 potassium channels. Patients' antibodies bind to the extracellular region of Caspr2 possibly abrogating Caspr2-Contactin-2 interaction. Caspr2 = contactin-associated protein-like 2. PSD = postsynaptic density protein. VGKC = voltage-gated potassium channel.

This review focuses on adult patients, but also highlights the pediatric cases. The first and second section cover the clinical syndromes associated to antibodies to LGI1 and Caspr2. Most physician have limited experience with these relatively new diseases, while recognition is essential because the diseases are treatable. Patient recognition should be based on clinical features, as the value of ancillary testing is often limited. The third section focusses on the subgroup of VGKC-positive patients without antibodies to LGI1 and Caspr2. The aim of this review is to improve the recognition of diseases associated to LGI1 or Caspr2 antibodies, and to clarify misconceptions regarding the clinical relevance of VGKC-test results.

LGI1-antibodies

Introduction

While LGI1-antibodies were discovered only six years ago, it is probably the most common cause of limbic encephalitis, and the second most common cause of autoimmune encephalitis after anti-NMDAR encephalitis. The clinical presentation of anti-LGI1 encephalitis is similar in most patients. (Box 1) Recognizing this clinical picture is essential, because routine ancillary testing can be non-specific.[11]

Pathogenesis

Unlike other epilepsy-related proteins, LGI1 is not a structural component of a receptor or ion channel, but a protein secreted by neurons. LGI1 forms a trans-synaptic complex with the presynaptic proteins ADAM11 and ADAM23 and postsynaptic ADAM22 and is involved in synaptic transmission excitability (Figure 1).[12,13] Pre-synaptically, ADAM23 interacts with the Kv1 subunit of the VGKC, and is essential for localizing Kv1.1 and kv1.2 subunit complexes to the synaptic terminals.[14] Post-synaptically, ADAM22 interacts with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) via PSD95 which is responsible for fast excitatory synaptic transmission, and necessary for hippocampal long-term synaptic plasticity (Figure 1A). During postnatal development, LGI1 mediates dendrite pruning and presynaptic and postsynaptic maturation in the hippocampus.

In humans, genetic disruptions of the LGI1-protein have been linked to autosomal dominant partial epilepsy with auditory features (ADPEAF).[15,16] In these patients, the LGI1 protein is not secreted or is unstable and fails to bind ADAM22.[17] The truncated mutant LGI1 prevents normal postnatal maturation of presynaptic and postsynaptic functions.[13]

In mice, LGI1 is widely expressed in inhibitory and excitatory neurons, but most extensively in the hippocampus. LGI1 null mice develop a lethal epileptic phenotype and most die by the third postnatal week. The lack of LGI1 disrupts synaptic connections and reduces AMPAR-mediated synaptic transmission in the hippocampus. This epileptic phenotype can be rescued by transgenic re-expression of LGI1.[18] Heterozygotic mutants show lowered seizure thresholds.[18] Mutated ADAM22 has been found to cause seizures as well.[17]

The pathogenesis of patients' LGI1 antibodies has been investigated recently. LGI1-antibodies associated with limbic encephalitis inhibit ligand-receptor interaction between LGI1 and ADAM22/23 by targeting the epitempin (EPTP) repeat domain and leucine-rich repeat (LRR) domain of LGI1. As a consequence, the number of synaptic AMPAR clusters is reduced in rat hippocampal neurons similarly to epileptic LGI1 knock-out mouse.[19] The increased excitability (manifested by seizures) in patients with LGI1 antibodies has been attributed to a predominant reduction of AMPARs in inhibitory neurons.[18] It is currently unclear whether patients' antibodies disrupt also the Kv1 potassium channels at the presynaptic level; this would result in increased excitability.

The exact mechanisms underlying the effects of patients' antibodies on the target, LGI1, are unknown. This is in contrast with the NMDAR or AMPAR antibodies occurring in patients with autoimmune encephalitis. These antibodies are IgG1 subclass and cause internalization of the corresponding receptors.[20,21] An interesting feature of anti-LGI1-encephalitis is that the antibodies are IgG4,[22,23] which are hetero-bispecific (continuously undergoing half-antibody exchange) and less effective than IgG1 in

crosslinking and internalizing the target antigen. Therefore, it is likely that the mode of action of LGI1 antibodies is by interfering protein-protein interactions between LGI1 and ADAMs.

Pathological analysis of the uncus of one patient with anti-LGI1 encephalitis showed some T-cell infiltration (half of them CD8+ cytotoxic T-cells) and complement activation, while MRI follow-up showed progressive atrophy.[24] As IgG4-antibodies cannot activate complement, it is currently unknown how this process occurs, but both direct T-cell mediated toxicity and an additional IgG1-mediated immune response are potential alternatives in some of the patients.

Epidemiology

Anti-LGI1-encephalitis had an annual incidence of 0.83/million in the Dutch population in 2015.[11] The incidence rate is rising, probably due to better recognition. Two-third of the patients are male. The disease usually occurs at age 50-70, but it may start as early as the third decade of life.[5,6,11,22,25-30]

Clinical syndrome

The vast majority of the patients present with limbic encephalitis (~90%), but some patients develop Morvan syndrome or a fragmented syndrome with only seizures or encephalopathy.[5,6,11,22,25,26] Common initial symptoms are seizures and cognitive disturbances. Disease usually progresses to a maximum severity in three to six months. During the course of disease, almost all patients experience disturbance of memory and behavior, often with spatial disorientation, and 90% develop seizures.[11,22,31] Three types of seizures occur. (Table 1) Typical of LGI1-encephalitis are faciobrachial dystonic seizures (FBDS), which occur in 26-71% of the patients.[11,22,30] FBDS are short-lasting (<30 seconds) dystonic contractions of an arm, often accompanied by ipsilateral facial contraction, or less frequently the ipsilateral leg.[26] FBDS occur 10-100 times a day and often start a few weeks before onset of cognitive decline. Second, subtle focal seizures occur in two-thirds of the patients, usually early in disease course as well. These seizures have mostly cognitive or autonomic features and occur multiple times a day (median 12/day). They are easily missed, as patient's descriptions are often vague, such as 'thoughts being pulled away', 'a shiver' or 'piloerection'.[32] More easily recognized are tonic clonic seizures, present in 60% of the patients and usually occurring only a few times during the severe stage of the disease.[11,31] The recognition of subtle seizures is essential in the diagnosis of LGI1-encephalitis, and diagnosis is even more challenging in the small group of patients without seizures. Subacute onset of cognitive dysfunction with spatial disorientation, insomnia, hyponatremia and signs of limbic encephalitis on MRI brain should be a clue for antibody testing. Interestingly, a small minority of patients with LGI1-antibodies present with Morvan syndrome.[11,23] These patients have both central and peripheral nervous system symptoms, although the LGI1 protein is thought to

be present in the central nervous system only. There is no clear explanation for the occurrence of peripheral nerve hyperexcitability, but the co-existence of other relevant autoantibodies against yet unknown antigens should be considered. Half of the anti-LGI1 patients have a sleep disorder, mostly insomnia, and autonomic dysfunction.[11,22] Symptoms of rapid-eye movement (REM) sleep behavior disorder were demonstrated in patients with limbic encephalitis and high titer VGKC-complex antibodies, subsequently proven to be LGI1 antibodies (Dr.F.Graus, personal communication).[33]

Table 1. Seizure subtypes in anti-LGI1 encephalitis

	Faciobrachial dystonic seizures	Focal seizures	tonic clonic seizures
Description	Short unilateral dystonic contraction of the arm (and face / leg)	Subtle cognitive or autonomic features	Generalized tonic-clonic seizures with loss of consciousness
Incidence	40-50%	60-70%	60-70%
Onset	Usually before cognitive symptoms	Usually before or simultaneous with cognitive symptoms	Usually at maximum disease severity
Frequency at maximum disease	40-100 per day	~ 10 per day	~ 3 during total disease course
Abnormalities on EEG during seizures	Not detectable is most patients[a]	Epileptic discharges	Epileptic discharges

[a] Electrodecremental events preceding the motor movement have been described[34], as well as a slow frontal wave, contralateral and preceding the FBDS.[31]

Ancillary testing

Routine ancillary testing can be contributive, although results are often non-specific. Mild to moderate hyponatremia is present in 60-74% of the patients.[5,6,11,22] Routine CSF is usually normal, or shows a slightly increased cell count. Brain MRI shows unilateral or bilateral hyperintensities in the medial temporal lobes in most patients, but the MRI is normal in 10-25%.[11,22,35] Basal ganglia hyperintensity on T1 and/or T2 sequences was reported in up to 42% of the patients with FBDS, and not in patients without FBDS.[36] However, this feature was seen in 1/16 patients with FBDS in a recent German study,[35] similar to our cohort (A.van Sonderen/M.Titulaer, unpublished data). Almost half of the patients develop mesial temporal sclerosis during the follow up.[11] Hippocampal atrophy was reported in 40-95% of patients.[11,35] This large difference might be partially explained by case selection and differences in treatment. EEG shows focal slowing or epileptic discharges in half of the patients. Interestingly, the majority of the FBDS have no ictal EEG correlates but longer FBDS can be preceded by electrodecremental events, usually preceding the onset of movement by approximately 500 msec,[11,34,37] or a slow frontal wave, contralateral to the FBDS.[31] In contrast, most EEGs show epileptic discharges during dyscognitive or autonomic focal seizures.[11,34] Variable tumor types are seen in 0-11% of the patients (Table 2).[5,6,11,22,28,30]

Antibodies can be detected in both serum and CSF, using cell-based assay (CBA) or brain tissue immunohistochemistry (Figure 3A). Due to absence of intrathecal antibody synthesis,[38] serum antibody testing might be more reliable. Accordingly, one study showed a higher sensitivity for serum analyses compared to CSF (100% vs 88%). The sensitivity for the commercial CBA with CSF was even lower (53%).[11] In contrast, another recent study using in-house CBA combined with immunohistochemistry showed a higher sensitivity with CSF (100% vs 92%).[22] Serum with LGI1-antibodies is positive on the VGKC-RIA and the titers are usually high.[11]

Box 1: Clinical characteristics of anti-LGI1 encephalitis

Patient
Male (67%) 50-70 years (can be younger)
Clinical syndrome: limbic encephalitis (90%)
Seizures (90%) - Faciobrachial dystonic seizures (FBDS, 50%) - Subtle focal seizures (65%) - Tonic clonic seizures (65%) Cognitive decline - Memory disturbance (97%) - Behavioral disturbance (90%) - Spatial disorientation (50%) Insomnia (65%)
Ancillary testing
Hyponatremia: 65% MRI brain: mesial temporal lobe hyperintensity: 75% CSF normal: 75% Tumor: <10%

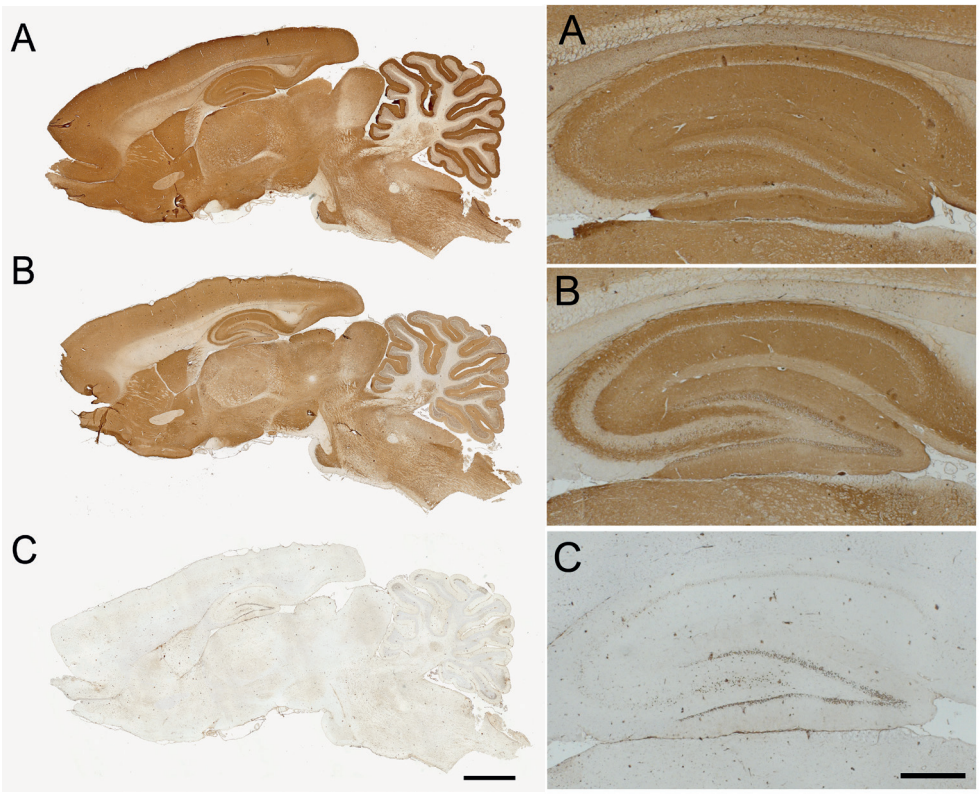

Figure 3. Immunohistochemistry on rat brain. Whole brain (left) and magnification of the hippocampus (right). (A) LGI1-antibodies cause staining of the hippocampus, excluding the inner one-third of the dentate gyrus, resulting in a typical pale strip. (B) Caspr2-antibodies show diffuse staining of the hippocampus. (C) Negative control, no staining.

Treatment and outcome

Anti-epileptic drugs usually have little effect on seizures, while immunotherapy shows impressive results.[11,25,30,36,39] To our experience, frequent FBDS or focal seizures almost disappear within hours to days after starting methylprednisolone. In patients presenting with FBDS, early immunotherapy is associated with preventing progression to limbic encephalitis.[39] The proposed therapeutic scheme (Figure 4) is based on cohort studies and expert opinion, as no therapy trials have been performed. First-line treatment consists of corticosteroids, intravenous immunoglobulin, plasma exchange or a combination of these. This treatment is reported to be effective in 50-80% of the patients.[11,22] While early response on seizure frequency is common, cognitive improvement usually takes months to a year. In a study, early treatment was associated with less severe cognitive outcomes.[35] For sustained improvement, pulse therapy is often followed by oral treatment with corticosteroids, azathioprine or mycophenolate mofetil. Little is known about the response to second-line treatment after first-line treatment failure. Rituximab has been considered particularly effective in IgG4-mediated disorders.[40] In a small series of 5 patients with anti-LGI1 encephalitis, rituximab was effective in 2 patients.

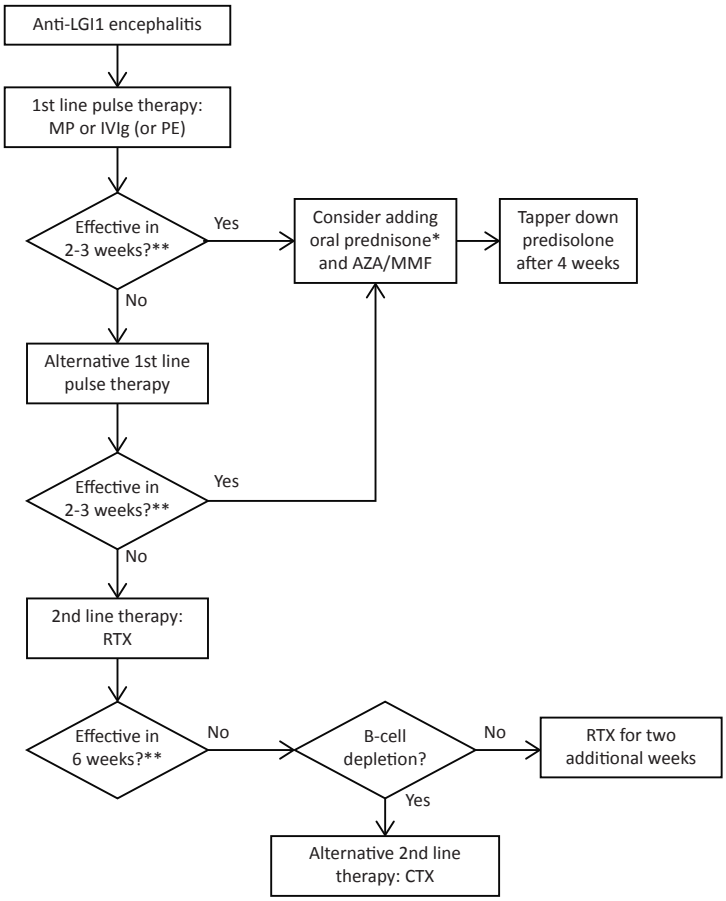

Figure 4. Proposed therapy scheme for anti-LGI1 encephalitis
*add oral prednisone in patients with moderate or severe disease. **expect early recovery of seizures, cognitive response may take longer. MP = intravenous methylprednisolone (3 days 1000 mg/day or 5 days 500 mg/day). IVIg = intravenous immunoglobulins (5 days 0.4 g/kg/day). PE = plasma exchange (3-6 sessions, alternate days). RTX = rituximab (4 weeks 375mg/m2 weekly). CTX = cyclofosfamide (6 months 750 mg/m2 monthly). AZA = azathioprine (usual dosage 2 dd 75 mg), MMF = mycophenolate mofetil (2 dd 720 mg).

However the series was too small to draw conclusions and the treatment delays were very long.[41] In a recent study, 70% of the patients had a favorable outcome (independent for activities of daily living) at follow up ≥2 years.[11,22] In most patients but not all, antibody tests became negative after recovery. Failure to respond to first-line therapy and clinical relapses were associated with poor outcome. The initial antibody level and the persistence of antibodies were not related to outcome.[22] The most frequent residual symptoms included memory deficits, apathy, and difficulties with spatial orientation. The latter

Table 2: Overview of studies with anti-LGI1 patients

Study	Additional inclusion criteria	No. of patients	VGKC titer median (range) pM	Male	Age median (range)	Syndrome	Tumor	Treatment effective	Good outcome (mRS 0-2)	Relapses
Irani, 2010[5]	VKGC > 400 pM	55†	NP	67%	NP	LE 89% MoS 4% Epilepsy 2%	0%	Mostly successful	34/39 (87%)	NP
Lai, 2010[6]	VGKC > 100 pM & LE	57	1054 (105-7600)	65%	60 (30-80)	LE 100%	11%[a]	NP	39/50 (78%) good outcome	6/33 (18%) median FU 1.5 years
Irani, 2011[26]	FBDS (26/29 LGI1)	29†	1962 (639-5409)	66%	64 (36-83)	LE 90%	0%	Mostly successful	NP	NP
Paterson, 2013[29]	NA	8	NP	63%	65 (18-86)	LE 63% PNH 25% PD 13%	NP	5/5 (100%)	NP	NP
Olberg, 2013[28]	NA	10	1196 (140-5143)	80%	64 (53-74)	LE 80% NMT 10% AIDP 10%	10%[b]	NP	NP	NP
Irani, 2013[39]	FBDS (9/10 LGI1)	10	1483 (346-4515)	50%	68 (29-92)	(Cognitive disorder 80%)	NP	100%	10/10 (100%) (mRS 0-1)	4/10 (40%) median FU 1.5 years
Shin, 2013[30]	NA	14	NP	57%	61 (41-78)	(Seizures 100% Cognitive disorder 86%)	7%[c]	11/12 (92%) minor or major improvement	11/12 (92%)	2/4 (50%) FU 1 mo – 2 yr
Rocamora, 2014[32]	Pilo-erectile seizures	3	NP	67%	39 (35-52)	Pilo-erectile seizures	NP	2/3 (67%) clear effect	2/3 (67%)	NP
Irani, 2014[41]	Rituximab	5	NP	20%	65 (48-73)	NP	NP	Rituximab: 2/5 (40%) minor or major effect	NP	NP
Malter, 2014[38]	LE	9	(440-7655)	67%	55 (32-67)	LE 100%	0%	8/9 (89%)	NP (8/9 seizure free; 6/8 remaining cognitive deficits)	0/9 (0%) (median FU 2 years)
Flanagan, 2015[36]	FBDS	26	400 (70-3460)	65%	62.5 (37-78)	LE 81%	8%[d]	18/18 (100%)	NP	NP
Steriade, 2016[37]	Epilepsy monitoring	9	NP	56%	54 (15-80)	NP	0%	NP	NP	NP
Gao, 2016[25]	NA	10	NP	70%	51.5 (27-75)	LE 90%	0%	9/9 (100%)	At 10 months: 9/10 (90%)	3/10 (30%) FU 2-30 mo
Li, 2016[27]	NA	10	NP	60%	Mean 58 (34-78)	(Seizures 100% Cognitive disorder 40%)	10%[e]	9/10 (90%)	9/10 (90%)	0/9 (0%) mean FU 10.2 months
Van Sonderen, 2016[11]	NA	38	720 (245-1314)	66%	64 (31-84)	LE 90% MoS 8% Epilepsy 3%	11%[f]	24/30 (80%)	>2 years: 67%	6/17 (35%) Median FU 42 mo
Arino, 2016[22]	NA	76	NP	66%	Mean 61 (32-80)	LE 83%	7%[g]	24/48 (50%)	At 2 years: 34/ 48 (71%)	13/48 (27%) Median FU 39 mo
Finke, 2017[35]	NA	30	NP	63%	Mean 66	LE 97% FBDS 3%	10%[h]	NP	24/30 (80%)	NP

NP = not provided. NA = not applicable. FBDS = faciobrachial dystonic seizures. LE = limbic encephalitis. MoS = Morvan syndrome. PNH = peripheral nerve hyperexcitability syndrome. NMT = neuromyotonia. AIDP = acute inflammatory demyelinating polyradiculoneuropathy. PD = Parkinson's disease. FU = follow up. †8 patients overlap.
[a]Tumor types: [a] thyroid (n = 2), lung, renal, ovarian, thymoma, [b] lip, thyroid, [c] renal, dprostate (n =2), [e]lung, fneuroendocrine pancreas tumor, thymoma, mesothelioma, rectum carcinoma in situ, [g]prostate, gastric neuroendocrine tumor, colon carcinoma, hneuroendocrine tumor jejunum, breast cancer, esophageal adenocarcinoma.

was shown with neuropsychological assessment,[11] and recently confirmed in another study that also demonstrated impaired verbal memory.[35] Overall, 86% of the patients suffer from persistent amnesia of the disease period and several months preceding the disease. This symptom is often misinterpreted as persistent disease activity, and can be very stressful to patients and relatives. Case fatality rate is 6-19%, including patients in whom the disease was not recognized and treated. Initial studies, with short-term follow-up, indicated relapses in 0-18% of the patients.[6,30,38] However, recent studies with longer follow-up (>2 years) showed that 27-35% of the patients had relapses.[11,22] This brings into consideration the use of long-term or aggressive immunotherapy, although it is unknown whether this actually diminishes the risk for relapses.

Pediatric cases

LGI1- and Caspr2 antibodies were identified in one child in a series of 39 children with VGKC-complex antibodies.[42] However, cohorts of more than fifty children with acute encephalitis and over 400 children with epilepsy who were screened for LG1-antibodies did not identify any positive patients.[43-47] Therefore, LGI1-antibodies are very uncommon in children and, in general, screening in pediatric patients with epilepsy or encephalitis seems not indicated.

Caspr2-antibodies

Introduction

Compared with LGI1-antibodies that associate with a discrete number of well-defined syndromes, Caspr2-antibodies occur in association with a wider variety of clinical syndromes. Most of these syndromes show a substantial overlap of symptoms, reflecting the frequent involvement of central and peripheral nervous system (Box 2). Clinical data are limited, as this disorder is rare.

Box 2: Clinical characteristics of Caspr2 disease

Patient
- Male (90%) - 60-70 years
Caspr2 core symptoms
- Cerebral symptoms (cognition 80%, epilepsy 50%) - Cerebellar symptoms (35%) - Peripheral nerve hyperexcitability (55%) - Autonomic dysfunction (45%) - Insomnia (55%) - Neuropathic pain (60%) - Weight loss (60%)
Ancillary testing
- MRI brain: normal (70%) - CSF: normal (75%) - Tumor: 20% (mostly thymoma)

Pathogenesis

Caspr2 is a cell adhesion molecule that belongs to the neurexin IV superfamily. The Caspr2 protein is encoded by the CNTNAP2 gene, located on chromosome 7q35. Together with TAG1, Caspr2 forms a transmembrane axonal complex present in the central and peripheral nervous system. These complexes cluster Kv1.1 and Kv1.2 of the VGKC at the juxtaparanodes of myelinated axons via PSD95 (Figure 2).[48,49] Axonal excitability is regulated by stabilizing conduction at the nodes of Ranvier, avoiding repetitive firing and helping to maintain the internodal resting potential.[50,51] In addition, Caspr2 is widely expressed by inhibitory neurons in the CNS, linked presynaptically to TAG1/Contactin and post-synaptically to Gephyrin. There it may function as a cell recognition molecule essential for synaptic network formation.[52]

Caspr2-deficient mice show mis-localization of Kv1.1/1.2, [48,49] alterations in the migration of cortical neurons, and a reduction in the number of GABAergic interneurons.[52] This is associated with epileptic phenotypes and autism-related behaviors. RNAi-mediated knockdown of Caspr2 affects synaptic organization and function in culture.[53] In humans, genetic disruption as well as point mutations or deletions in the target domains are associated with intellectual disability, seizures and autistic features.[54]

Patients' antibodies are directed to the Caspr2-extracelullar domain. The N-terminal Discoïdin and Laminin G1 modules of Caspr2 have been identified as target epitopes of autoantibodies.[55,56] Neither the 3-dimensional structure nor a single subdomain is absolutely necessary for antibody binding.[55] Antibodies target inhibitory interneurons in the hippocampus, potentially disrupting the interaction of Caspr2 with TAG1, indirectly altering the Gephyrin clustering post-synaptically.[56] Caspr2-antibodies are of the rare IgG4 subtype, just as LGI1-antibodies.[22,57] IgG4 subtype does not mediate complement activation, and do not bind Fc receptors on effector cells. Therefore, Caspr2-antibodies may be pathogenic by blocking the function of the targets or protein-protein interaction, not by internalization or complement-mediated toxicity. One case report mentioned some complement deposition, although the clinical relevance in a disease in which IgG4 antibodies predominate remains questionable.[58]

Epidemiology

Caspr2-antibody mediated disease has a strong male predominance (90%), for which there is no explanation yet. Age at onset is around 60-70 years, but female patients tend to be younger.[5,57,59,60] The disease is rare, with approximately 150-200 patients encompassing all reported series.

Clinical syndrome

The majority of the patients develops limbic encephalitis or Morvan syndrome,[5,57] but there is substantial overlap in the main symptoms. Seven core symptoms have been

identified. (Box 2) These symptoms can be subtle and not mentioned by patients or family members spontaneously. Half of the patients develop seizures and 80% show cognitive deficits. Over half of the patients have peripheral nerve hyperexcitability. In this disease, spontaneous muscle activity results in myokymia, fasciculations and muscle cramps. Hyperhydrosis is a common expression of autonomic dysfunction. Other common features are burning pain in the extremities, cerebellar symptoms, insomnia and weight loss. The disease often progresses for a few months, but progression over one year is not uncommon (~30%). Therefore, Caspr2-antibody-associated disease potentially mimics a neurodegenerative disease, mainly in patients with prominent cognitive decline.[57] Although one study suggested the presence of Caspr2-antibodies in 10% of patients with idiopathic cerebellar ataxia[61], the index case developed Morvan syndrome and only 3 of the other 7 patients sera showed reactivity with brain tissue; in our experience patients with idiopathic cerebellar ataxia (without the core symptoms indicated above) do not develop Caspr2-antibodies (J.Dalmau/M.Titulaer, unpublished data).

Ancillary testing

Standard laboratory examination is usually normal. Mildly raised CSF cell count or protein can be detected, but CSF is unremarkable in many patients (~75%). Brain MRI is usually normal, but (bilateral) hyperintensity of the medial temporal lobe can be present.[57,60,62] EEG results are non-specific. 20% of the patients have a tumor, mostly thymoma and more frequently in patients with Morvan syndrome or neuromyotonia.[57] A recent study showed that the co-existence of antibodies against Caspr2 and Netrin receptors (DCC and UNC5a) associated with thymoma.[63]

Antibodies can be detected with brain tissue immunohistochemistry and specifically confirmed with a CBA. The antibodies show a diffuse hippocampal staining on rat brain tissue. (Figure 3B) Confirmation can be achieved with combining serum and CSF analysis, or with the combination of CBA with immunohistochemistry or immunocytochemistry with cultured live neurons. Ninety percent of Caspr2-antibody positive samples test positive in the VGKC-RIA (median titer 414 pM; positive > 100 pM).[57,60] Since anti-Caspr2 encephalitis is rare and the specificity of serum CBA testing is high, but not perfect, additional testing of CSF or the use of confirmatory tests should be encouraged.[57,64] A study suggested that the clinical manifestations varied according to the presence or absence of antibodies in CSF (e.g., absent antibodies in patients with neuromyotonia and Morvan syndrome, and present in patients with limbic encephalitis).[59] Our experience is different; we found antibodies in CSF of patients with any type of anti-Caspr2 associated encephalitis, including limbic encephalitis, neuromyotonia and Morvan syndrome,[57,65] and therefore it seems reasonable to examine serum and CSF in all patients.

Treatment and outcome

Therapy trials have not been performed in patients with anti-Caspr2 associated syndromes, but the majority of the reported patients were treated with immunotherapy. the most frequent treatments included steroids, intravenous immunoglobulin or a combination of both, and some patients additionally received second-line treatment (rituximab and/or cyclophosphamide). In several cohorts, response to treatment was from 79% to over 90%.[57,60,62,64] Successful tumor treatment seems essential in patients with a malignancy.[57] The largest reported series indicated a favorable outcome (modified Rankin Scale ≤2) in 73% of the patients, and the two-year fatality rate was 10%. Relapses occurred in 25% of the patients, sometimes up to six years after the initial disease episode. Interestingly, relapses can present with symptoms similar to those of the first episode of other Caspr2 core symptoms (Box 2).[57]

Table 3: Overview of studies with anti-Caspr2 patients

Study	No. of patients	VGKC positive (%); median titer (range)	Male	Age Median (range)	Syndrome	Tumor	Treatment effective	Good outcome (mRS 0-2)	Relapses
Irani, Brain 2010[5]	19	100% (inclusion > 400 pM)	84%	NP	37% LE 37% NMT 16% MoS 11% Epilepsy	32%[a]	Non-tumor patients improved	11/17 (65%)	NP
Lancaster, 2011[60]	8	86%	88%	60.5 (46-77)	NP	0%	7/7 (100%)	7/8 (88%)	NP
Malter, 2014[38]	3	100% 1419 pM (509-2061)	100%	47 (38-69)	NP	0%	2/3 (67%)	NP (2/3 seizure free; 1/2 cognitive deficits)	0/3 (0%) FU 11-58 mo
Sunwoo 2015[62]	5	NP	60%	43.5 (8-65)	60% Epilepsy 20% MoS	NP	4/4 (100%)	4/5 (80%)	NP
Joubert, 2016[59]	18[b]	NP	94%	64.5 (53-75)	78% LE	17%[c]	Mostly effective	12/16 (75%)	6/16 (38%) FU 6-114 mo
Van Sonderen, 2016[57]	38	89% 414 pM (50-815)	89%	66 (25-77)	42% LE 29% MoS 13% PNH 8% Cerebellar	19%[d]	25/27 (93%)	24/33 (73%)	7/28 (25%) FU 1-14 yr
Bien, 2017[64]	20[e]	75% (101-705 pM)	100%	63 (33-75)	75% LE 15% MoS 5% LE + ataxia 5% MD	10%	11/14 (79%)	13/17 (76%)	NP

NP = not provided. LE = limbic encephalitis. NMT = neuromyotonia. MoS = Morvan syndrome. PNH = peripheral nerve hyperexcitability syndrome, MD movement disorder. mRS = modified ranking scale. FU = follow-up. Tumor types: [a] thymoma (n = 5), endometrial adenocarcinoma, [b] 33 patients were included, but only in only 18 detailed clinical details could be extracted,[c] prostate adenocarcinoma plus chronic lymphoid leukemia, thyroid cancer (n = 2), [d] thymoma (n = 4), lung adenocarcinoma (n = 2, one without biopsy) , carcinoma in situ of sigmoid, [e] 62 patients were included; only 27/62 were described in detail; two had no IgG antibodies; 5 no CSF tested or confirmatory testing. Additional patients identified in cohort screening without extensive clinical description are provided in Supplemental material Table S1.

Pediatric patients

Several cohorts of pediatric patients were screened for Caspr2-antibodies. In most cases results were negative,[43-45] and the essential laboratory confirmation was lacking in the majority of the Caspr2-CBA positive cases. Screening in patients with epilepsy resulted in 4/178 Caspr2 positive patients, of which two were positive on hippocampal neuronal culture.[47] Three of 114 children with new-onset epilepsy were Caspr2 positive. Remarkably, these samples were VGKC-RIA negative and no additional laboratory confirmation was reported.[46] One of 23 patients suspected to have autoimmune encephalitis were Caspr2 positive on the CBA.[66] One of 39 patients with VGKC-complex antibodies had antibody-specificity for both LGI1 and Caspr2, and also showed reactivity with cultured neurons; this 3-year old boy had Guillain-Barré syndrome.[42] We identified Caspr2 antibodies in a 9-years old girl with epilepsy and autonomic dysfunction, confirmed with brain immunohistochemistry and immunostaining of hippocampal cultures of neurons (M. Titulaer, unpublished data). In conclusion, Caspr2-antibodies do occur in children, but this is very rare and should always be confirmed with additional laboratory tests.

VGKC-positivity in the absence of LGI1 and Caspr2 antibodies

Introduction

Since 1995, VGKC-complex antibodies have been determined with RIA. In 2010, it was found that the antibodies were not directed to the potassium channel itself, since no reactivity was seen with VGKC-transfected cells. Currently, the clinical relevance of VGKC-positivity in the absence of LGI1 and Caspr2 antibodies is unclear.

Patients with VGKC-complex antibodies lacking LGI1 and Caspr2 antibodies can present with limbic encephalitis or neuromyotonia,[7,67] but also with many other manifestations. Indeed, a wide clinical spectrum of VGKC-complex antibody positivity (in the absence of antibodies to LGI1 and Caspr2) has emerged in recent years. For example pain, in combination with other neurological symptoms or as sole manifestation, was reported to be associated with VGKC-complex antibodies.[68] Similarly, these antibodies were reported in patients with pathology-proven Creutzfeldt-Jakob disease,[69] and many other diseases such as psychogenic non-epileptic seizures, REM sleep behavior disorder, multiple system atrophy, peripheral neuropathy, vasculitis, seizures, mitochondrial disease, periodic paralysis, hepatic encephalopathy, frontotemporal lobar degeneration, Lewy body disease, Asperger syndrome, or schizophrenia.[28,29,70-73] This prominent lack of syndrome specificity and detection of high titer VGKC-complex antibodies even in cases without autoimmune disease have raised concern for misdiagnosis, unnecessary immunotherapies, and in general whether these antibodies have any clinical utility.[74]

Clinical relevance

Hundreds of patients have been reported in studies analyzing the clinical relevance of VGKC-complex antibodies. However, data regarding patients with VGKC-complex antibodies lacking antibodies to LGI1 and Caspr2 is very limited, for several reasons. First, subgroups with and without antibodies to LGI1 and Caspr2 are usually lumped together, even in recent studies (Suppl. Table S3). In 2016, a study reported a higher incidence of epilepsy in 62 patients with VGKC-complex antibodies compared to controls. Determination of LGI1 and Caspr2 antibodies was not performed despite that patients with these antibodies frequently have seizures.[75] In another study, Klein and colleagues[68] reported a high incidence of pain in 316 VKGC-complex antibody positive patients, mainly those with Caspr2-antibodies. Although the authors mentioned studies for LGI and Caspr2 antibodies, these results were not included. Analysis of pain characteristics and outcome was provided for the entire group irrespective of antibody results. Another study reported the MRI findings in 42 patients with VGKC-complex antibodies; 33 of them were tested for antibodies to LGI1 (22 positive) or Caspr2 (3 positive), but this sub-classification was not considered in the analysis of the results.[76]
Several other studies analyzed the relevance of VGKC-complex antibody positivity according to the titers of antibodies. Huda et al. reported the difference in clinical syndrome between patients with a titer <400pM and >400pM. Unfortunately, antibodies to LGI1 and Caspr2 were only analyzed in a small number of patients, and these results were not taken into account in the main analysis.[70] Some studies compared the likelihood of an autoimmune etiology in patients with lower and higher titers. Olberg et al. compared 12 patients with a VGKC-complex antibody titer >500pM with 20 patients with a lower titer. They conclude that titers >500pM are most likely associated with autoimmune disease and response to immunotherapy. However, this conclusion was based on an analysis of data that included patients with LG1 and Caspr2 antibodies that are known to more often correlate with high titer VGKC-complex antibodies, and also are known to respond better to treatment.[28] Paterson and colleagues reported a definite autoimmune disorder in 3/32 patients with a low VGKC-complex antibody titer (<400pM) and in 10/23 patients with a high titer (>400pM). Again, patients with antibodies to LGI1 and Caspr2 were included in the analyses, thereby clouding the value of titers of VGKC-complex antibodies lacking LGI1 and Caspr2-antibody specificity. Interestingly, VGKC-complex antibody titers of almost 1000pM were seen in patients with frontotemporal lobe degeneration and Lewy body dementia, both very unlikely to be autoimmune.[29] Apart from lumping together cases with VGKC-complex antibodies with or without LGI1 and Caspr2 specificities, the reported series are heterogeneous as cut-off values differ between laboratories.

Several series conclude that the clinical relevance of VGKC-positivity is supported by the favorable response to immunotherapy. For example, symptoms improved after immunotherapy in 8/10 patients with pain syndromes and in 3/4

patients with seizures.[68,77] However, these patients are not compared to untreated cases or to patients without VGKC-complex antibodies with similar syndromes. Therefore, the natural course of the disease, regression to the mean, and the non-immunological effects of corticosteroids were not taken into account. In summary, data from all these studies contain many confounding factors and prevent from drawing conclusions on the clinical value of VGKC-complex antibodies (without LGI1 or Caspr2 antibodies).

In 2016, the clinical relevance of antibodies to VGKC-complex was analyzed in a case-control study. 25 patients without LGI1 and Caspr2 antibodies were compared to 50 negative patients, matched by age, gender and clinical syndrome. Results showed that both groups were comparable with regard to MRI abnormalities and CSF findings. Assessed blindly, based on predefined criteria, there was no difference in the proportion of patients with evidence of autoimmune inflammation in both groups (28% vs 18%, p=0.18). There was no cut-off value for the VGKC-titer that was found useful to discriminate patients with and without autoimmune inflammation. Comparing the matched VGKC-complex antibody positive and negative patients, tumor incidence (28% vs 21%, p=0.73) and response to immunotherapy (50% vs 82%, p=0.19) were not different.[71]

In this study patients with a neurologic disorder suspected to be autoimmune who were found positive for VGKC-complex antibodies (but were LGI1 and Caspr2 antibody negative) had the same likelihood to have an autoimmune disorder than dose who had the same clinical syndrome and were negative for VGKC-complex antibodies. It has been argued that patients without LGI1 and Caspr2 antibodies may have antibodies against a yet unknown target, but so far this target has not been found and patients serum or CSF samples do not show reactivity with neuronal cell surface antigens (J.Dalmau, unpublished data). A group of investigators suggested that contactin-2 could be one alternative target. Antibodies directed to contactin-2 were found in 5 patients, of whom three had co-existing LGI1 or Caspr2 antibodies; clinical descriptions were not provided.[5] The same investigators reported 3 additional patients with antibodies to contactin-2 among 178 children with epilepsy; two of them had an autistic spectrum disorder.[47] Similar antibodies have been found in patients with multiple sclerosis[78] and the clinical significance in cases with VGKC-complex antibodies has not been reproduced by other investigators.

Future research might identify other more plausible targets in patients with VGKC-complex antibodies who are negative for LGI1 and Caspr2 antibodies. Of course the study by Van Sonderen and colleagues will need confirmation by others, but in the meantime, we recommend caution with the interpretation of positive VGKC-complex antibody results. This test by itself is unreliable to establish a clinical diagnosis and formulate a treatment plan. In patients with VGKC-complex antibodies that are double negative for LGI and Caspr2 antibodies the clinical syndrome and ancillary studies should prevail over the positive VGKC-complex antibody findings.[74,79]

Pediatric cases

While controlled studies in adults are limited, useful data regarding pediatric cases are even scarcer. VGKC-complex antibodies without antibodies to LGI1 and Caspr2 were detected in 1/46 children with acute encephalitis,[44] 4/10 patients with encephalitis with prominent seizures,[45] and 3/124 children with focal epilepsy.[43] Four of 10 children with criteria for limbic encephalitis had VGKC-complex antibodies (not tested for LGI1 or Caspr2 antibodies).[80] Interestingly, the response to immunotherapy was disappointing in most of the patients. In contrast, a series of 12 patients with VGKC-complex antibodies, without determination of specificity for LGI1 or Caspr2 antibodies, described partial or full recovery in all treated patients; as a result of this study, the authors recommended prompt and aggressive immunotherapy to all patients with VGKC-complex antibodies.[81] These patients had a wide variety of symptoms, including motor tics, painful feet, dysarthria and global developmental regression, making it unlikely that all could be attributed to one disease. In addition, treatment results were not compared to a control group, and should therefore be interpreted with caution. In a recent series of 39 children with VGKC-complex antibodies and many different syndromes, the authors concluded that these antibodies cannot be used as a positive predictive value for clinical management, although an association between high titer of the antibodies and inflammatory disease was suggested by the ICD10-coding of the syndromes. The authors suggested that VGKC-complex antibodies are nonspecific biomarkers for (primary or secondary) inflammation.[42]

In conclusion, current data are insufficient to draw firm conclusions about VGKC-positivity in children. In line with studies in adult patients, VGKC-complex antibodies by themselves are unreliable to establish a clinical diagnosis and formulate a treatment plan.

Table 4. Overview of studies with VGKC-positive patients lacking antibodies to LGI1 and Caspr2

Study	No. of patients	Male gender	Age mean (range)	Syndrome	Treatment effective	Comparison to VGKC-negative matches Conclusion
Suleiman, 2011[45]	4	25%	11 (1-14)	Encephalitis with seizures	NP	No
Olberg, 2013[28]	19	53%	60 (19-88)	11% LE 89% other	NP	No
Paterson, 2013[29]	43	53	56 (18-80)	7% LE 5% MoS 5% Epilepsy 2% NMT 81% other	6/7 (86%) improved‡	No
Malter, 2014[38]	6	50%	52 (19-72)	100% LE	NP (3/6 seizure free; 2 with cognitive deficits improved, but remained with deficits)	No
Hacohen, 2015[42]	39	NP	Pediatric	47% encephalopathy 11% epilepsy 16% ADS 26% other	14/20 (70%) improved‡	No
Lahoria, 2016[72]	3	NP	61 (60-71)	Peripheral neuropathy	1/1 (100%) improved‡	No
Van Sonderen, 2016[71]	25	44%	55 (1-79)	28% LE 12% PERM 4% epilepsy 4% NMT 8% psychosis 44% other	6/12 (50%) improved‡	Yes. VGKC-positivity did not seem to be a marker for autoimmunity.

NP = not provided. LE = limbic encephalitis. MoS = Morvan syndrome. NMT = neuromyotonia. ADS = acquired demyelinating syndrome. PERM = progressive encephalomyelitis with rigidity and myoclonus. ‡Many patients remained untreated; selection bias.
Additional patients identified in cohort screening without extensive clinical description are provided in Supplemental material Table S2.

Conclusion

In recent years, major advances have been made in the field of VGKC-complex related antibodies. Antibodies to LGI1 and Caspr2 were discovered in 2010 and are very likely to be pathogenic. They are related to well-described clinical syndromes. Recognizing these syndromes is essential, as these patients are treatable with immunotherapy with a favorable prognosis. Tumor screening is indicated in these patients. There is no convincing evidence that VGKC-complex antibodies in the absence of antibodies to LGI1 and Caspr2 are specific markers for autoimmune neurological diseases. As all these autoantibodies are very infrequent in children, even more caution should be taken to formulate diagnostic and treatment decisions in the pediatric population.

Key points

- Three groups of VGKC-positive patients should be distinguished: patients with antibodies to LGI1, patients with antibodies to Caspr2 and patients lacking these antibodies.
- Patients with LGI1 antibodies usually present with typical limbic encephalitis, including alteration of memory, behavior, spatial disorientation and several types of seizures.
- Patients with Caspr2 antibodies present with various syndromes involving the central and/or peripheral nerve system, mainly including cognitive decline, epilepsy and peripheral nerve hyperexcitability.
- Patients with antibodies to LGI1 or Caspr2 usually respond well to immunotherapy.
- The clinical relevance of antibodies against VGKC-complex, but without reactivity with LGI1 and Caspr2, is uncertain; in these patients, clinical assessment and ancillary tests prevail for the establishment of a diagnosis and formulation of treatment.

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Supplemental material

Table S1: Patients with Caspr2 antibodies identified in cohort screening (no extensive clinical description provided)

Study	Cohort	Caspr2 positive patients	Antibodies confirmed[a]	Male	Age Median (range)
Suleiman, 2013[1]	Pediatric new-onset epilepsy (n= 114)	n =3	No	67%	7 (0.5-10)
Ekizoglu, 2014[2]	Patients with focal epilepsy (n = 81)	n = 4	No	25%	37 (35-40)
Baysal-Kirac, 2016[3]	Patients with autonomic seizures (n = 58)	n = 5	Yes, 4/5 confirmed[b]	20%	30 (15-36)
Vanli-Yavuz, 2016[4]	Patients with epilepsy and hippocampal sclerosis (n = 111)	n = 11	No	18%	17 (0-49)
Wright, 2016[5]	Pediatric epilepsy (n = 178)	n = 4	Yes, 2/4 confirmed[c]	100%	9.7 (0.6 – 12.5)

[a]Antibody confirmation with addition laboratory techniques or CSF analysis
[b]Commercial CBA followed by in-house CBA and immunohistochemistry
[b]In house CBA followed by staining with live hippocampal neurons

Table S2: VGKC-positive patients without LGI1 and Caspr2 antibodies; identified in cohort screening (no extensive clinical description provided)

Study	Cohort	VGKC positive patients (LGI1 and Caspr2 neg)	Antibodies confirmed[a]	Male
Suleiman, 2013[1]	Pediatric new-onset epilepsy (n= 114)	n = 4	75%	3 (1.5-4.9)
Brenner, 2013[6]	Epilepsy (n = 416)	n = 19	63%	NP
Hacohen, 2013[7ǂ]	Pediatric autoimmune encephalopathy (n = 48)	n = 7	NP	8.9 (6-15)
Hacohen, 2014[8]	Pediatric acute demyelinating disorder (n = 65)	n = 3	67%	9 (6-14)
Baysal-Kirac, 2016[3]	Patients with autonomic seizures (n = 58)	n = 3	67%	26 (24-57)
Vanli-Yavuz, 2016[4]	Patients with epilepsy and hippocampal sclerosis (n = 111)	n = 4	50%	6 (0-13)
Borusiak, 2016[9]	Pediatric focal epilepsy (n = 124)	n = 3	33%	5 (2-6)
Wright, 2016[5]	Pediatric epilepsy (n = 178)	n = 3	33%	4 (1.7-7.4)
Hacohen, 2016[10]	Pediatric brainstem encephalitis (n = 57)	n = 3	NP	NP

ǂ Overlap with Hacohen et al, Neurology 2015. NP = not provided

Table S3: VGKC-positive patients, analyzed (predominantly) irrespective of LGI1 and Caspr2 antibody result

Study	Additional inclusion criteria	No. of patients	Antibody classification	Male	Age Median (range)	Syndrome	VGKC titer median (range)	Treatment effect	FU and outcome
Haberlandt, 2011[11]	Pediatric LE	4	NP	25%	14 (3-16)	LE	180 (134 – 310)	NP	1/4 (25%) recovered, 1/4 (25%) died
Dhamija 2011[12]	Pediatric	12	NP	33%	7.5 (8 mo-14 y)	Diverse	NP	7/7 (100%) minor/major improvement*	NP
Cornelius, 2011[13]	NA	15	NP	60%	56 (17-80)	33% LE 27% MoS	1,510 (90-4,860)	9/11 (82%) improved	NP
Somers, 2011[14]	NA	152	NP	45%	59 (2-87)	44% neuropsychiatric symptoms	150 (30-14,500)	11/21 (52%) improved *	NP
Jaben, 2012[15]	PE	5	NP	40%	59 (46-76)	80% LE 20% NMT	(80-2062)	3/5 (60%) improved	NP
Irani, 2012[16] ǂ	MoS	29 (23 VGKC positive)	6/27 Caspr2 3/27 LGI1 15/27 LGI1+Caspr2	93%	57 (19-80)	MoS (38% thymoma)	NP	17/27 improved (63%)	31% died
Klein, 2012[17] ǂǂ	NA	316	53/316 LGI1 36/316 Caspr2	NP	NP	Diverse Pain in 50%	NP	13/16 (81%) pain reduction *	NP
Lilleker, 2013[18]	Seizures & Titer>400pM	6	1/6 LGI1 1/6 Caspr2	83%	Mean 52.5 (27-77)	Seizures	(180-3450)	6/6 (100%) improved	NP
Baumgartner, 2013[19]	LE	7	1/7 LGI1 2/7 Caspr2	71%	61 (26-71)	LE	NP	NP	NP
Frisch, 2013[20]	LE	15	NP	53%	57 (38-73)	LE	NP	NP	Majority improved
Sarkis, 2014[21]	LE	4	NP	75%	59 (29-80)	LE	NP	NP	NP
Liewluck, 2014[22]	CFS	9	2/7 LGI1 1/7 Caspr2	89%	45 (12-62)	CFS	NP	3/4 (75%) improved *	NP
Kotsenas, 2014[23]	Seizures	42	22/32 LGI1 3/32 Caspr2	52%	56 (8-79)	Seizures	NP	NP	NP
Huda, 2014[24]	NA	57	3/19 LGI1 2/19 Caspr2	56%	59 (25-77)	21% LE 37% PNH 2% MoS 40% Other	251 (101-4064)	20/23 (87%) improved *	NP
Urbach, 2015[25]	LE	36	16/36 LGI1 6/36 Caspr2	61%	Mean 58 (19-86)	LE	NP	NP	NP
Wagner, 2016[26]	LE	16	5/16 LGI1 5/16 Caspr2	63%	55 (19-72)	LE	1,318 (427-7,655)	NP	NP
O'Sullivan, 2016[27]	NA	62	NP	NP	NP	Diverse	NP	NP	NP

NP = not provided. NA = not applicable. LE = limbic encephalitis. PE = plasma exchange. MoS = Morvan's syndrome. CFS = cramp fasciculation syndrome. NMT = neuromyotonia. PNH = peripheral nerve hyperexcitability syndrome.
ǂ Three patients also described in Irani et al, Brain 2010
ǂǂ Klein JAMA Neurol 2013 excluded due to substantial overlap in patients with Klein Neurology 2012.
*Many patients remained untreated; selection bias.

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PART II:

The postsynaptic glutamate receptors

CHAPTER 7

Encephalitis and AMPA receptor antibodies: novel findings in a case series of 22 patients

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Abstract

Objective

We report the clinical features, co-morbidities, and outcome of 22 newly identified patients with antibodies to the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR).

Methods

This was a retrospective review of patients diagnosed between May 2009 and March 2014. Immunological techniques have been previously reported.

Results

Patients' median age was 62 years (range 23-81; 14 female). Four syndromes were identified: 12 (55%) patients presented with distinctive limbic encephalitis (LE), eight (36%) with limbic dysfunction along with multifocal/diffuse encephalopathy, one with LE preceded by motor deficits, and one with psychosis with bipolar features. Fourteen patients (64%) had a tumor demonstrated pathologically (5 lung, 4 thymoma, 2 breast, 2 ovarian teratoma) or radiologically (1 lung). Additional antibodies occurred in seven patients (3 onconeuronal, 2 tumor-related, and/or 3 cell-surface), all with neurological symptoms or tumor reflecting the concurrent autoimmunity. Treatment and outcome were available from 21 patients (median follow-up 72 weeks, range 5-266): five had good response to immunotherapy and tumor therapy, 10 partial response, and six did not improve. Eventually five patients died, all had a tumor or additional paraneoplastic symptoms related to onconeuronal antibodies. Coexistence of onconeuronal antibodies predicted a poor outcome (p=0.009).

Conclusion

Anti-AMPAR encephalitis usually manifests as LE, can present with other symptoms or psychosis, and is paraneoplastic in 64% of the cases. Complete and impressive neurological improvement can occur, but most patients have partial recovery. Screening for a tumor and onconeuronal antibodies is important because their detection influences outcome.

Introduction

The recent characterization of autoimmune synaptic disorders has led to the identification of subtypes of limbic, multifocal or generalized encephalitis that often respond to immunotherapy. One of the antibodies targets the GluA1 or GluA2 (previously called GluR1 or GluR2) subunits of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), an ionotropic receptor that belongs to the family of glutamate receptors. AMPAR mediates most of the fast excitatory synaptic transmission in the brain, and is important for synaptic plasticity, memory, and learning.[1] The initial description of the encephalitis associated with these antibodies was published in 2009 and included 10 patients, all with limbic encephalitis (LE) who had CSF and serum antibodies that reacted with the neuropil of rat brain and the cell surface of cultures of rat hippocampal neurons, leading to precipitate and characterize the target antigens as the GluA1 or GluA2 subunits of the AMPAR.[2] Recent studies have shown that these antibodies cause a selective decrease in the total surface amount and synaptic localization of GluA1 and GluA2-containing AMPAR through increased internalization and degradation[3], resulting also in a decrease of AMPAR-mediated currents[3, 4]. Since the initial description of this disorder, only a few cases with similar antibodies have been reported and therefore the clinical manifestations are largely unknown.[5-8] We report 22 additional patients and describe the clinical presentation, cancer-association, response to treatment, co-morbidities, prognostic factors, and outcome.

Materials and Methods

Patients

Sera or CSF of 10,573 patients with suspected autoimmune encephalitis or paraneoplastic neurological syndromes (including LE, non-focal encephalitis, encephalomyelitis, psychiatric disorders, dementia, Morvan syndrome, and cerebellar dysfunction) were included in the studies of antibody screening. The samples were received between May 2009 and March 2014 in the Department of Neurology, University of Pennsylvania, Department of Neurology, Erasmus Medical Center, Rotterdam, and the Center of Neuroimmunology at Institut d´Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona. Clinical information was obtained by the investigators or from questionnaires completed by the referring physicians and telephone interviews. One patient was previously published as isolated case report.[9]

Standard protocol approvals, registrations, and patient consents

Informed consent for antibody studies was obtained in all patients. The study was approved by the Institutional Review Boards of the Hospitals of the University of Pennsylvania, Erasmus Medical Center Rotterdam, and University of Barcelona.

Screening for antineuronal antibodies

Serum and CSF samples were tested for antibodies to intracellular and cell surface antigens using brain immunohistochemistry on rat brain as previously reported.[2], 10 Samples showing specific tissue staining were further examined with in-house or immunoblot assays for antibodies to onconeuronal antigens (Hu, Yo, Ri, CV2, amphiphysin, Ma1/2, Tr), tumor-associated antigens (SOX1, ZIC4), and non-tumor-associated antigens (GAD65, AK5, Homer3).[11,12] The identity of the target cell- surface or synaptic autoantigens was determined with HEK293 cells expressing LGI1, CASPR2, NMDAR, AMPAR, and GABA(B)R, as reported.[2,13-16] The type of AMPAR subunit identified by patients´ antibodies was investigated using HEK293 cells expressing only the GluA1 or GluA2 subunits of the receptor.[2] We did not include in these investigations the determination of antibodies using immunoblot of recombinant, denaturated AMPAR subunits,[4] given that these antibodies do not produce the typical neuropil immunostaining of the antibodies studied here and their syndrome association remains to be established.

Review of previously reported cases of tumor-associated anti-AMPAR encephalitis

In order to assess the significance of the presence of an underlying tumor or additional paraneoplastic antibodies in the clinical outcome (survival and relapse frequency), we reviewed the current data along with all previously reported oncological or paraneoplastic cases of anti-AMPAR encephalitis.[2,5,6,8]

Statistical Analysis

Age between groups was compared by Mann-Whitney U test, time until diagnosis by Student t test, and comparison of serum and CSF by McNemar test. For survival, Kaplan-Meier curves were created, using log-rank tests. The association of relapses with type of therapy (aggressive, nonaggressive) was compared with Fisher exact test. Statistical significance was defined as p-value less than 0.05.

Results

Patients

We identified 22 patients with AMPAR antibodies. A summary of the clinical information is shown in Tables 1 and 2. The median age was 62 years (interquartal range [IQR] 37.3-70.3 years; range 23 to 81 years) with no differences between patients with or without tumor (60.5 years, IQR 38.8-70.3, and 64 years, IQR 35-72, respectively; p=0.80). The female: male ratio was 14:8. Fourteen patients (64%) had an underlying tumor; in 13 the tumor was demonstrated pathologically, including five small-cell lung cancer (SCLC), four thymoma, two breast cancer, and two ovarian teratoma, and in another patient the tumor was demonstrated radiologically (lung cancer). One patient

had onconeuronal antibodies (CRMP5), but no tumor has been identified until now (30 weeks follow-up).

The median time from symptom onset until diagnosis of autoimmune encephalitis was 6.5 weeks (IQR 4-18.3 weeks), and was not different between patients with or without tumor (5.5 weeks [IQR 3.8-8.8] vs 13 weeks [5-52], respectively; p=0.11). In 12 patients the neurological symptoms occurred before tumor diagnosis or led to tumor screening (median 5 weeks [IQR 2.3-37.5], range 1.5 to 56 weeks) and in two patients the tumor was identified before developing encephalitis (1 year and 6 months, respectively). The CSF analysis showed lymphocytic pleocytosis in 11/22 patients (median leukocyte count 23 cells/ml, range 5 – 820), and elevated protein concentration in 10/20 (median 71 mg/dl, range 49 – 425); only 5/22 patients had an elevated protein concentration as isolated finding (Table 1).

Table 1. Clinical presentation in patients with AMPAR autoimmune encephalitis.

N°	age/ sex	Symptom onset until diagnosis (weeks)	Clinical presentation (initial symptoms)	Other symptoms during course of the disease	Initial MRI	EEG	CSF	Additional antibodies
1	42/F	6	LE	mild hemiparesis left	bilateral increased signal in temporal lobe	epileptiform activity (clinically no seizures)	normal WBC and protein	-
2	51/F	2	LE	-	increased signal in left medial temporal lobe	NA	normal WBC and protein	-
3	59/M	17	LE	-	bilateral increased signal in temporal lobes	normal	6 WBC, 50 mg/dl protein	-
4	62/M	7	LE	hemiparesis, mutism	bilateral increased signal in temporal lobe and insula	NA	normal WBC and protein	-
5	63/F	4	LE with seizures	hyponatremia	increased signal in medial temporal lobes	NA	normal WBC, 425 mg/dl protein	GABA(B)R SOX1
6	70/F	8	LE	-	normal	epileptiform activity (clinically no seizures)	normal WBC, 64 mg/dl protein	-
7	81/F	4	LE	hyponatremia	bilateral increased signal in temporal lobe	focal spike waves; polymorphic delta left fronto-temporal	normal WBC and protein	SOX1
8	33/F	9	LE with seizures	-	bilateral increased signal in hippocampi and amygdalae	epileptiform activity	14 WBC, normal protein	-
9	35/M	2	LE	-	increased signal in medial temporal lobes	focal activity temporal	23 WBC, proteins NA	-
10	64/F	52	LE	-	increased signal in left medial temporal lobe	diffuse slowing and focal abnormalities	5 WBC, normal protein	-
11	72/F	22	LE	spasticity	increased signal in medial temporal lobes	general slowing	50 WBC, 49mg/dl protein	-
12	72/F	13	LE	-	bilateral increased signal in temporal lobe	slow (theta) activity	52 WBC, 100 mg/dl protein	-
13	23/M	3	short-term memory loss, seizures, psychosis	catatonia, decerebrate posturing right arm	patchy increased signal in cortex of both hemispheres and basal ganglia	general slowing and epileptiform activity left temporal lobe.	23 WBC, normal protein	-
14	25/F	2	psychosis, confusion, agitation, non-verbal, seizures, dyskinesias	fever, hypertension, required intubation	normal	NA	normal WBC and protein	NMDAR

15	53/F	5	confusion, bradypsychia, status epilepticus, autonomic dysfunction	-	increased signal in medial temporal lobes, frontobasal, and caudate	NA	164 WBC, 92 mg/dl protein	CRMP5
16	65/F	52	short-term memory loss, confusion, abnormal behavior, itching, and involuntary arm movement	seizures	hyperintensities in the corpus callosum	normal	Normal WBC, 71 mg/dl protein	-
17	71/M	5	somnolent, seizures, disoriented, tremor	-	abnormality in the hypothalamic region with mass effect on pituitary gland; T2/FLAIR increased signal in right temporal	generalized slowing	normal WBC, elevated protein	NMDAR
18	72/M	6	short-term memory loss, ataxia, insomnia, psychotic features	sensory polyneuropathy	normal	normal	Normal WBC, 65 mg/dl protein	Amphi
19	62/M	26	short-term memory loss, confusion, and abnormal behavior, psychosis, optic neuropathy	insomnia, ataxia	hyperintensities in basal ganglia	focal activity	33 WBC, 173 mg/dl protein	CRMP5
20	69/M	52	seizures, short-term memory loss, confusion, psychosis, aphasia	hyponatremia, fatigue, weakness, ataxia; later Parkinsons disease	increased signal in medial temporal lobes, cortical parietal, cingulum, frontal	lateralized periodic slowing temporal and hippocampi	normal WBC and protein	-
21	29/F	11	left sided weakness, spasticity	LE, psychosis, dysarthria, tachycardia, hypertension	increased signal in insula, putamen, and thalamus	generalized slowing of background, focal slowing in right hemisphere	13 WBC, normal protein	-
22	38/F	5	psychosis with bipolar features	nystagmus, anti-psychotic induced-neuroleptic malignant syndrome, fencing posturing, autonomic dysfunction	normal	normal	90 WBC, protein NA	-

Amphi = amphiphysin antibodies; FLAIR = fluid-attenuated inversion recovery; LE = limbic encephalitis; NA = not available; WBC = white blood cells.
Clinical presentation of LE defined by the presence of short-term memory loss, confusion, and abnormal behavior

Clinical presentation and MRI features

Four clinical presentations were identified: 12 patients (55%) developed symptoms of LE, defined by the presence of short-term memory loss, confusion, and abnormal behavior (Table 1, patients 1-12). Two of these patients developed seizures (nos. 5 and 8) and one of them (no. 5) had additional GABA(B)R and SOX1 antibodies. Hyponatremia occurred in two patients, both with SCLC. In 11 of the 12 patients, the clinical diagnosis of LE was confirmed by the MRI findings of unilateral (2 patients) or bilateral (9 patients) mesiotemporal increased fluid-attenuated inversion recovery (FLAIR)/T2 signal abnormalities (Figure 1 A and B). In patient 6 (Table 1), the brain MRI was normal.

Eight patients (Table 1, cases 13-20) had diffuse encephalitis with clinical and/or MRI evidence of involvement of multiple areas of the CNS (Figure 1 C and D). All developed limbic dysfunction along with one or more of the following symptoms: 6 had seizures, 5 prominent psychiatric manifestations, 3 ataxia, 2 abnormal movements, and 1 each, optic neuropathy and aphasia. Hyponatremia occurred in one patient without cancer. Two patients in this group had NMDAR-antibodies (further described in Immunological studies).

Another patient (Table 1, patient 21) was a 29-year-old woman, who first presented with left sided weakness and spasticity involving face, arm, and leg, and two months later developed memory loss, confusion, abnormal behavior, psychiatric symptoms, visual hallucinations, autonomic dysfunction, and dysarthria. The MRI showed increased FLAIR/T2 signal in the right thalamus, bilateral putamen, and cerebellum. Tumor screening revealed an ovarian teratoma but NMDAR-antibodies were negative.

The remaining case (patient 22, table 1) was a 38-year-old woman who presented with new onset psychosis with bipolar features. She was started on anti-psychotic medication and one week later developed nystagmus and neuroleptic malignant syndrome (rigidity, tonic fencing-like posture, and autonomic instability), requiring intubation for airway protection. The brain MRI was normal, EEG demonstrated moderate generalized slowing, CSF showed 90 leukocytes/mm3, and the tumor screening was negative. Her mental status did not improve and she was lost to follow-up after 5 weeks.

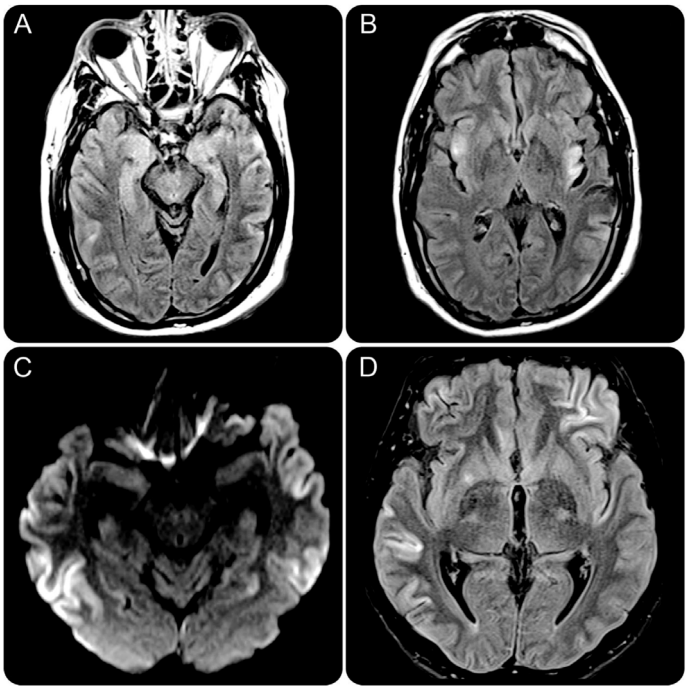

Figure 1. Brain MRI findings of anti-AMPAR encephalitis.
Brain MRI obtained 6 weeks after symptom onset (patient 4, table 1) shows increased T2/fluid-attenuated inversion recovery (FLAIR) signal abnormalities involving medial temporal lobes (A) and insular cortex (B). In addition, the frontal and parieto-occipitial cortex and claustrum show mild focal hyperintensity (B). Diffusion-weighted images of patient 13 (table 1) show widespread involvement of the temporal cortex (C). Similar abnormalities are shown on FLAIR sequences involving the left frontal and right temporal cortex as well as the right putamen (D).

Immunological studies

All patients´ serum and/or CSF samples showed intense neuropil staining on brain tissue immunohistochemistry and reacted with HEK cells co-expressing GluA1/2 subunits of the AMPAR2. From 5 patients only CSF was available and from another 3 only serum was available; paired samples were available from 14 patients, all CSF were antibody positive but only 10/14 sera were positive (p=0.13). Four patients had antibodies only against the GluA1 subunit, 7 only against the GluA2 subunit, and 9 against both subunits (Figure 2); in 2 cases the limited amount of sample did not allow for independent subunit assessment. There were no significant differences among clinical presentation, association with a tumor, or prognosis in patients with antibodies recognizing different subunits.

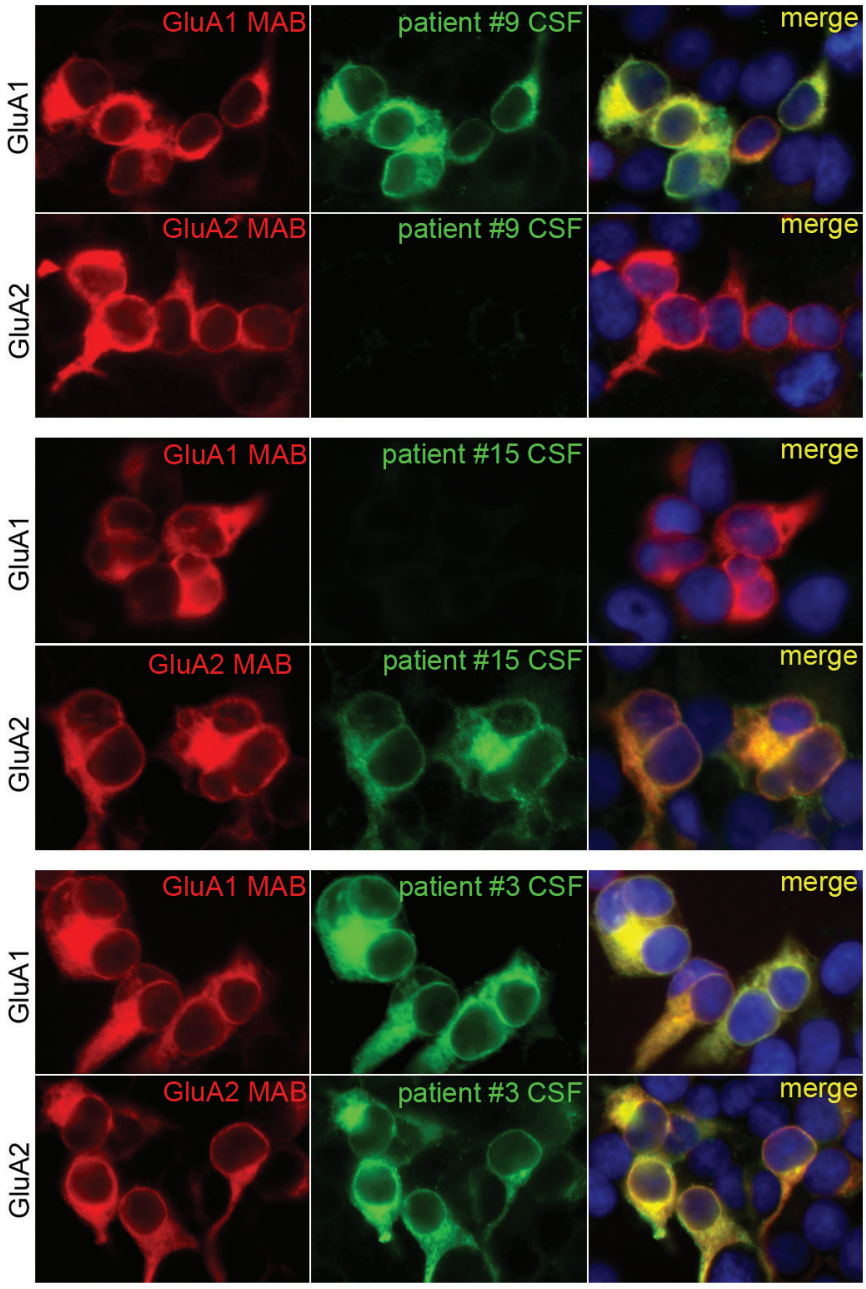

Figure 2. Reactivity of patients´antibodies with GluA1 or GluA2 subunits of the AMPAR.
Patients´antibodies were identified on HEK293 cells transfected with GluA1 or GluA2 subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). Examples of patients with antibodies targeting only GluA1 (patient 9), or GluA2 (patient 15), or both subunits (patient 3) are shown. Patients´antibodies are shown with green fluorescence; commercial monoclonal antibodies against GluA1 or GluA2 are shown with red fluorescence; the blue nuclear staining is shown with 4',6-diamidino-2-phenylindole (DAPI). All panels: x 400.

Additional neuronal antibodies were found in 7 patients (3 onconeuronal-intracellular, 1 SOX1 [tumor-associated], 2 cell surface-synaptic, and 1 SOX1 and cell surface/synaptic), 6 of them with an associated tumor or cancer (table 2). The three patients with onconeuronal antibodies (2 CRMP5, 1 amphiphysin) developed a clinical phenotype, outcome, or tumor association that were more characteristic of the additional immune response than that of the AMPAR-antibody syndrome (tables 1 and 2). Two of the 3 patients with additional antibodies to cell-surface/synaptic proteins [1 GABA(B)R, 2 NMDAR] developed syndromes influenced by the additional antibody: the patient with GABA(B)R-antibodies had seizures and SCLC, both typical of this autoimmune syndrome, and one patient with NMDAR-antibodies developed psychosis, confusion, agitation, decrease of verbal output, seizures, and dyskinesias, all typical of anti-NMDAR encephalitis. In contrast, the second patient with NMDAR-antibodies was atypical in many respects, including demographics (71-year-old man), symptoms (somnolence, seizures) and tumor (thymic carcinoid). The presentation with somnolence is atypical of NMDAR autoimmunity, and it was likely related to an inflammatory lesion in the hypothalamus (table 1); this case probably represents an overlap syndrome of 2 or more autoimmune disorders.

Treatment and follow up

Clinical follow-up was available from 21 patients (median 72 weeks, range 5 to 266). The remaining patient (patient 22) was lost to follow-up shortly after diagnosis. Twenty patients received first-line immunotherapy (steroids, IV immunoglobulins, or plasma exchange) and 5 of them also second-line immunotherapy (rituximab, cyclophosphamide) (table 2). Thirteen patients had one or more of the following oncological treatments: 7 tumor resection, 8 chemotherapy, and 6 radiation therapy (table 2). Five patients showed good neurological response to immunotherapy or oncologic therapy with a modified Rankin Scale (mRS) score between 0 and 1 at the last follow-up; 10 patients had partial response with an mRS score between 2 and 3, and 6 patients had poor or no response to treatment (table 2). One of the patients with partial response (patient 11, table 2) relapsed 2 months later; this patient was lost to follow-up after relapse. Of the 6 patients with poor or no response to treatment, 4 had a malignant tumor or additional antibodies (1 SOX1, 1 amphiphysin, and 2 CRMP5) with superimposed paraneoplastic symptoms (see details in supplemental information).

There was no significant change in survival for patients with or without a tumor (median 123 weeks vs not met, all 6 without a tumor still alive; p=0.086; figure 3A; the patient with onconeuronal CRMP5-antibodies but without tumor was excluded from this as well as from the following analysis). On the other hand, the median survival of the 4 patients with tumor and onconeuronal or SOX1-antibodies (cases 5, 7, 15, and 18) was 52 weeks (range 5 – 123 weeks; all died), while that of the 10 patients with

tumor but without additional onconeuronal antibodies was not met (median follow-up 61 weeks, range 10 – 266 weeks; 9 of 10 alive at last follow-up; p=0.009; figure 3B).

In order to further assess the significance of the presence of an underlying tumor and onconeuronal antibodies in patient outcome, we reviewed the data of all oncological cases of the current study together with those previously reported.[2,5,6,8] Of 24 patients identified, 7 had onconeuronal, GAD65, or SOX1 antibodies and 17 had no additional antibodies. Six out of 7 patients with paraneoplastic syndromes and additional antibodies have died (median survival 65 weeks); in contrast, 15 of 17 patients with tumor, but without additional onconeuronal antibodies were alive (median survival not met; median follow-up 60 weeks, range 10 – 516 weeks; p=0.003). Eleven out of 13 patients without tumor are alive (median follow-up 104 weeks; range 25 to 390), which was not significant compared to patients with tumor (p=0.079; figure 3C).

To determine whether clinical relapses were associated with less aggressive therapy, we reviewed the data of 21 patients of the current study and those of 16 previously reported cases for which information was available.[2,5,6,8] Overall, relapses occurred in 6/37 patients (16%, 1 case in the current study). While 0/19 patients who received aggressive therapy (chemotherapy or rituximab) had relapses, 6/18 who did not receive aggressive therapy had relapses (p=0.008).

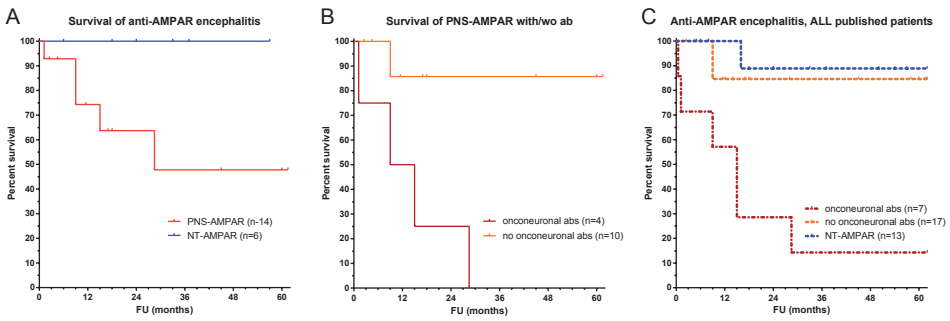

Figure 3. Survival of patients with paraneoplastic or idiopathic anti-AMPAR encephalitis.
(A) Kaplan-Meier survival curves for patients with paraneoplastic anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) encephalitis (with or without onconeuronal antibodies, red) and patients with idiopathic anti-AMPAR encephalitis (blue). (B) Kaplan-Meier survival curves for 4 patients with paraneoplastic anti-AMPAR encephalitis plus additional onconeural antibodies (dark red) and 10 patients with paraneoplastic anti-AMPAR encephalitis without onconeuronal antibodies (orange). (C) Kaplan-Meier survival curves for all assessable reported patients with paraneoplastic anti-AMPAR encephalitis and onconeuronal antibodies (dark red), paraneoplastic anti-AMPAR encephalitis without onconeuronal antibodies (orange) and idiopathic anti-AMPAR encephalitis (blue).[2,5,6,8]

Table 2. Treatment and outcome of patients with AMPAR autoimmune encephalitis.

N°	Tumor	Additional antibodies	Symptom onset until start of treatment (weeks)	Neurological outcome measuring mRS (compared with mRS at diagnosis)	immunotherapy or chemotherapy	Treatment response	Follow-up (weeks)
1	breast cancer	-	4	2 (5)	tumor resection, chemotherapy, IVIg	partial	195
2	SCLC	-	2	1 (3)	chemotherapy + radiotherapy, steroids	full	266
3	SCLC	-	11	1 (3)	chemotherapy + radiotherapy, IVIg	full	72
4	Malignant thymoma	-	8	1 (5)	tumor resection, steroids, IVIg	full	19
5	SCLC	GABA(B)R SOX1	13	2 (4)	chemotherapy + radiotherapy	partial	65, died of cancer
6	SCLC	-	8	2 (3)	chemotherapy + radiotherapy, steroids	partial	39, patient died of cancer
7	SCLC	SOX1	4	3 (3)	chemotherapy, steroids	no	123, patient died of cancer
8	-	-	13	3 (3)	steroids, plasmaexchange, rituximab, cyclophosphamide	no	160
9	-	-	2	2 (3)	steroids, IVIg, rituximab	partial	104
10	-	-	52	2 (3)	steroids, IVIg, plasmaexchange	partial	78
11	-	-	4	3 (5)	steroids, IVIg	partial / relpase	25
12	-	-	13	2 (3)	steroids	partial	142
13	thymoma	-	5	3 (5)	tumor resection, IVIg, steroids, rituximab	partial	10
14	ovarian teratoma	NMDAR	2	0 (5)	tumor resection, steroids, IVIg	full	50
15	malignant thymoma	CRMP5	1	5 (4)	tumor resection, chemotherapy, radiotherapy, steroids, IVIg	no	5, patient died of cancer
16	breast cancer	-	13	2 (3)	tumor resection, chemotherapy, radiotherapy, steroids, IVIg, plasmaexchange	partial	260
17	thymic carcinoid	NMDAR	3	1 (4)	tumor resection, steroids, plasma exchange	full	78
18	lung tumor	Amphi	4	5 (4)	steroids*	no	39, patient died of cancer
19	-	CRMP5	21	4 (3)	steroids, rituximab	no	30
20	-	-	52	3 (4)	IVIg, plasmaexchange	partial	247
21	ovarian teratoma	-	8	5 (5)	tumor resection; steroids, IVIg, plasmaexchange, rituximab	no	19
22	-	-	NA	NA (5)	NA	NA	5

Amphi = amphiphysin antibodies; IVIg = IV immunoglobulin; mRS = modified Rankin Scale; NA = not available; SCLC = small cell lung cancer Treatment response was defined by decrease of at least 1 score in the mRS with an mRS at the last follow-up < 3. At the last follow-up, all patients with good response had mRS of 0-1, and all cases with partial response had mRS of 2-3.
* patient died befor tumor diagnosis was confirmed.

Discussion

We report 22 newly identified patients with anti-AMPAR encephalitis and provide several clinically relevant findings including (1) the frequent presentation of the disorder as LE, sometimes with prominent psychiatric features, psychosis, or hyponatremia that can mislead the initial diagnosis, (2) the presence of an underlying tumor in 64% of the patients, (3) the coexistence of onconeuronal and cell surface autoantibodies in 32% of the patients, all with symptoms or tumors that reflect the concurrent autoimmunity, and (4) the frequent but limited response to treatment, with long-term outcome influenced by the presence of onconeuronal antibodies and related paraneoplastic symptoms or tumors.

The term LE refers to an inflammatory process of the brain restricted clinically or radiologically to the limbic system, which includes the medial temporal lobes and frontobasal and cingular regions. Despite a well-defined syndrome, it is frequently poorly recognized, misnaming as LE any type of autoimmune or paraneoplastic process above the foramen magnum. Among the many antigens of paraneoplastic and autoimmune encephalitis, there are three that typically associate with LE, including LGI1, GABA(B) R, and AMPAR.[2,13,16-18] At least 60% of the patients with any of these disorders develop a typical clinical or radiological picture of LE, with additional symptoms or associations that can suggest the antigen. For example, while the LE with LGI1-antibodies frequently associate with hyponatremia but almost never to SCLC[13], the LE with GABA(B) R-antibodies frequently occurs with early and prominent seizures and 50-60% of the patients have SCLC.[16,18] Recognition of each autoantigen is important because all 3 disorders are potentially treatable but for the appropriate treatment one should consider the associated comorbidities or tumors (further discussed later). Our findings show that LE with hyponatremia not only occurs in patients with LGI1-antibodies, but also in some patients with antibodies to the AMPAR or GABA(B)R, either as part of SCLC-related syndrome of inappriopriate antidiuretic hormone (which was not further investigated in 2 of our patients) or as a primary effect of the immune-response (one patient did not have cancer).

In the initial report of 10 patients with anti-AMPAR encephalitis the main findings were that all developed LE, 7 had a tumor (lung, breast, or thymoma), and the disorder frequently responded to treatment but had tendency to relapse. The current data confirm some of these findings and expand on others. Ten of the current patients did not initially present with classical LE, although most of them had limbic dysfunction concurrent or heralded by other symptoms. Importantly, 6 of these patients had prominent psychiatric symptoms, one of them manifesting as pure psychosis for 1 week before developing a neuroleptic malignant syndrome induced by treatment along with mild additional neurological symptoms. The presentation of anti-AMPAR encephalitis as pure psychosis (without additional neurological symptoms) has been previously

reported in 2 cases[6], indicating that this type of encephalitis should be included in the differential diagnosis of autoimmune psychosis.

The histological types of tumors were similar to those previously reported in anti-AMPAR encephalitis[2], but 2 patients had ovarian teratoma (one without evidence of NMDAR-antibodies), an association not previously reported. We do not know why patients in the current study had fewer neurological relapses than those in previous studies (1/21 versus 5/16);[2,5,6,8] however, the approach to therapy may be a reason. Indeed, when considering all current and previous cases for which treatment information is available (n = 37), all clinical relapses were in the group who did not receive aggressive therapy (6 of 18 patients, p=0.008). Aggressive treatment has been associated with lower relapse risk in anti-NMDAR encephalitis.[19] Another possible explanation could be a diagnosis bias: at the time of the initial study, patients with monophasic disease were often not sent for antibody testing and missed, resulting in an overrepresentation of relapsing patients.

Overall, 71% of the patients responded to immunotherapy or treatment of the tumor, most of them showing a partial neurological response (48%). These data suggest that patients with AMPAR antibodies have less substantial recoveries than those with other types of autoimmune encephalitis [NMDAR, LGI1 or GABA(B)R]. Despite this, substantial and sometimes unexpected recoveries do occur, but require aggressive therapy. An example is patient 13 who presented with severe encephalitis, refractory seizures, and a large thymoma, but his Karnofsky performance status was considered too low (30/100) for treatment. The patient was transferred to our Institution (Hospital Clinic, Barcelona) under pharmacologically induced coma and mechanical ventilation. The brain MRI showed changes suggestive of widespread cortical damage (figure 2). He underwent intensive immunotherapy and tumor removal, and currently is at home, free of seizures, and rapidly improving. This case suggests caution in clinically assessing autoimmune encephalitis using Karnofsky or similar neurological scales for treatment decisions.

The presence of concurrent antibodies, mainly onconeuronal (CRMP5, amphiphysin) or tumor biomarkers linked to paraneoplastic autoimmunity (e.g. SOX1), is associated with additional paraneoplastic symptoms and a poorer prognosis. The 5 patients who died had one or more of these features along with an underlying tumor. These findings along with the review of previously reported cases with tumor (either with or without an additional paraneoplastic neurological autoimmunity),[2,5,6,8] suggest that the presence of an additional paraneoplastic autoimmunity is the main prognostic factor for a poor outcome. Indeed, the survival of patients with tumor but without additional paraneoplastic autoimmunity was similar to that of patients without tumor, but the survival of patients with tumor and additional paraneoplastic autoimmunity was significantly worse than that of the other subgroups. Similar comorbidities linked to a poor prognosis have been reported in patients with anti-GABA(B)R encephalitis,

a disorder that frequently occurs with SCLC and may associate with concurrent paraneoplastic immune responses (e.g., amphiphysin, Ri, SOX1).[18]

This and previous studies suggest an interesting molecular link between the symptoms of LE and the effects of LGI1 and AMPAR antibodies. Indeed, LGI1 is a secreted neuronal protein that forms a trans-synaptic complex interacting at the postsynapse with ADAM22 and the AMPAR and at the presynapse with ADAM23 and the shaker potassium channel Kv1.[20] There is evidence that LGI1-antibodies prevent the binding of LGI1 to ADAM22 and by unclear mechanisms result in a decrease of AMPAR (the effects on the presynapse have not been determined).[21] On the other hand, the antibodies of patients with anti-AMPAR encephalitis bind directly to the GluA1/2 subunits causing a decrease of the levels of receptors by antibody-mediated internalization.[2] Therefore, 2 seemingly different immune responses against different synaptic proteins result in a common downstream effect (decrease of post-synaptic AMPAR clusters), providing a potential explanation for the frequent association of both disorders to classical LE.

The current findings taken together with those of previous studies have several practical implications. First, in patients with classic LE with or without tumor association, determination of AMPAR-antibodies should be considered. These antibodies may also occur in patients with multifocal encephalitis usually involving the limbic system, and less frequently can present as pure psychosis. Second, detection of GluA1/2 AMPAR-antibodies should lead to an extensive screening, including underlying tumors, classical paraneoplastic antibodies, and other cell surface antibodies. Third, prompt treatment of the tumor and immunotherapy are important, because the disorder is potentially reversible, but the outcome seems related to the presence of concurrent paraneoplastic autoimmunity linked to an underlying tumor.

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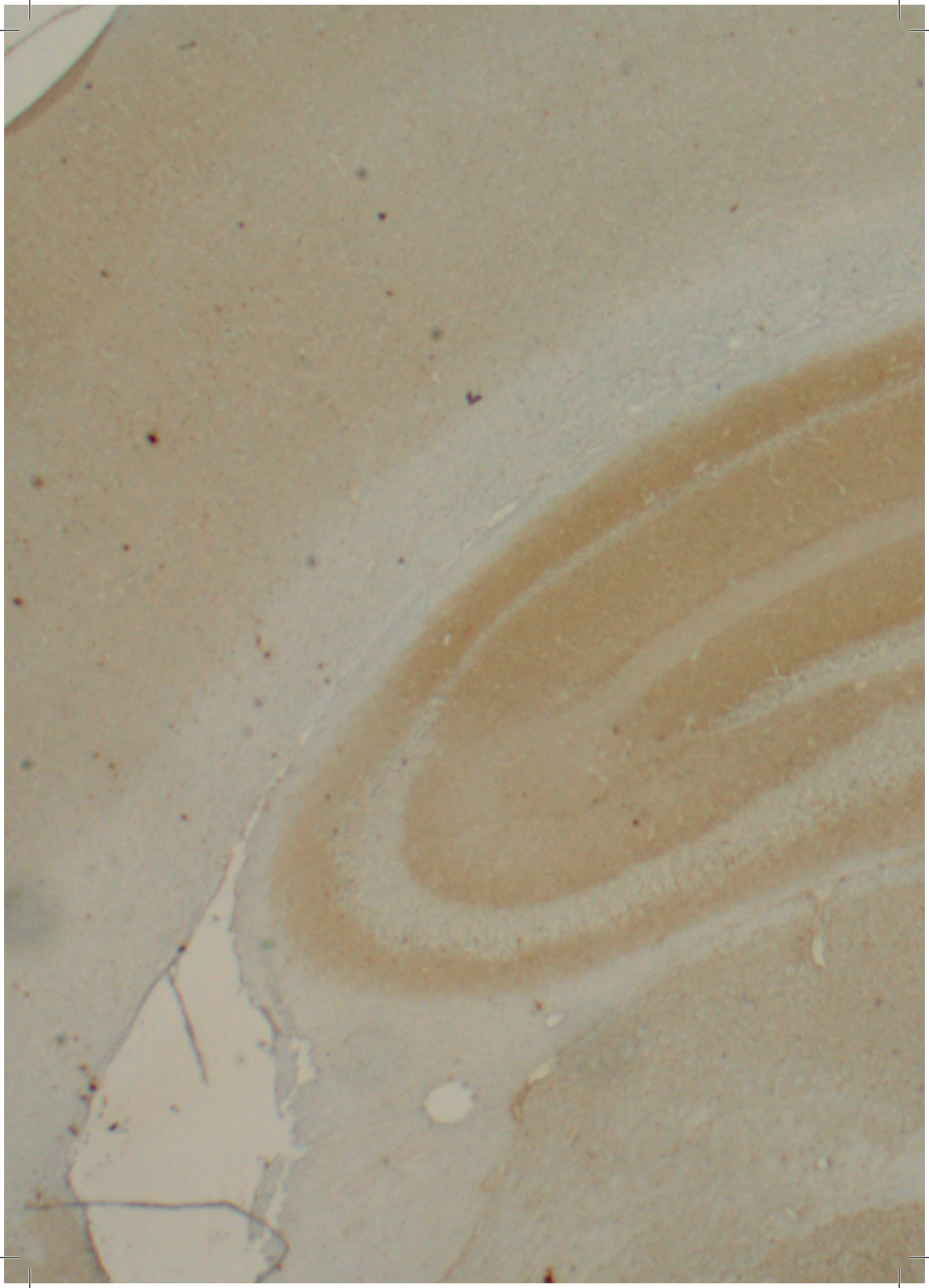

CHAPTER 8

Treatment considerations in a therapy-resistant protracted case of anti-NMDAR encephalitis

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Submitted

CHAPTER 9

The predictive value of electroencephalography in anti-NMDA receptor encephalitis

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Abstract

Objectives

Anti-N-methyl-D-aspartate receptor encephalitis (anti-NMDARE) is a severe, but treatable disease. This study aims to give a detailed description of electroencephalogram (EEG) results in pediatric and adult patients to improve disease recognition, and analyzes the predictive value of first EEG for final clinical outcome.

Methods

This nationwide cohort study includes patients with NMDA receptor antibodies confirmed with cell-based assay and immunohistochemistry, in serum and cerebrospinal fluid. EEG recordings were re-evaluated by two experienced neurophysiologists, mixed with control EEGs for blinding. Initial EEG as well as follow up registrations were analyzed.

Results

35 adults and 18 children were included. Only two patients (4%) had a normal EEG. During first recording, the majority of the patients had normal posterior rhythm (71%), which was associated with better modified Rankin Scale at final outcome (OR 4.74; 95%CI 1.56–14.47; p=0.006). In addition, EEGs showed focal (73%) or diffuse (67%) slowing. First EEG was severely abnormal in 26%. However, 8/14 patients with a severely abnormal first EEG still had a favorable outcome. During the course of disease, extreme delta brushes (EDB) were present in 6/53 (11%) patients.

Conclusions

First EEG commonly shows normal posterior rhythm with focal or diffuse slowing. Although sensitivity of an abnormal EEG is high (96%), normal EEG does not exclude anti-NMDAR encephalitis. EDBs are only present in severely affected patients. First EEG recording is predictive for final clinical outcome.

Introduction

Anti-N-methyl-D-aspartate receptor encephalitis (anti-NMDARE) is the most common antibody-mediated encephalitis. Patients develop subacute psychiatric symptoms, memory loss, movement disorders and seizures, often followed by intensive care unit (ICU) admission due to consciousness decline, autonomic dysfunction or hypoventilation.[1] Disease mostly affects women of childbearing age, or children. 38% of the patients have a tumor, mainly ovarian teratoma. Antibodies are directed to the NR1 subunit of the NMDAR, which is found across the brain. This explains why disease is not restricted to the limbic area. MRI brain is often normal, but electroencephalogram (EEG) is useful to analyze the functional deficits caused by the NMDAR-antibodies. EEG studies have reported diffuse slowing in a substantial part of the patients.[2,3] In a study of 9 children, diffuse abnormalities with lack of normal posterior rhythm was associated with poor outcome,[4] but this has not been studied further. Most larger studies aim to give a description of all disease characteristics and therefore do not analyze or report detailed EEG data.[1,2,5-9] Also, questions remain regarding the occurrence of extreme delta brushes (EDB). The pattern of EDB is pathognomonic for anti-NMDARE,[10,11] but incidence ranges from 0-100%, depending on patient selection, clinical situation and timing of EEG.[3,8,10,12] Our study aims to give a full description of EEG results in an unselected group of pediatric and adult patients with anti-NMDARE. First EEG as well as follow up registrations are analyzed, and the predictive value of EEG is discussed.

Methods

Patients accrual and laboratory testing

Samples had been sent for antibody testing to the laboratory of Medical Immunology of the Erasmus University Medical Center, Rotterdam. Samples were sent from July 2006 until July 2017. NMDAR-antibodies were detected with cell-based assay (Euroimmun, Lübeck, Germany) and immunohistochemistry on rat brain,[13,14] in both serum and CSF, if available. Patients were considered positive if at least two tests confirmed the presence of NMDAR-antibodies. Both adult and pediatric (< 18 years at disease onset) patients were included in this study if at least one EEG recording was available for analysis. Patients were included in the longitudinal part of the study if EEG recordings from predefined stages of disease were available: within 2 weeks after disease onset, after one month and after three months (at least 2 out of 3).

Clinical information was obtained from medical records, and included demographic data, clinical symptoms, tumor presence, intensive care admissions and treatment. First line immunotherapy included corticosteroids, intravenous immunoglobulin and/or plasma exchange. Second line treatment included cyclophosphamide and/or rituximab. Disease severity and clinical outcome were

measured with the Modified Rankin Scale (mRS). Outcome was analyzed if follow up was at least 6 months after disease onset. Outcome mRS ≤ 2 was considered favorable, as this reflect independency in daily life.

EEG analysis

EEG recordings were collected retrospectively. All recordings were based on the International 10-20 system and the majority of the recordings were spot EEG. In the few cases with continuous registration, only the first thirty minutes were included in the analyses. EEG recordings were independently re-evaluated by two experienced neurophysiologists (DT, SA), mixed with control EEGs for blinding. If in disagreement, both neurophysiologists convened to achieve agreement. In all patients, the first available EEG was evaluated. If serial EEGs were recorded, the EEG at disease nadir was analyzed as well. The following EEG characteristics were evaluated: posterior dominant rhythm, diffuse slowing (mild, moderate, severe), focal slowing, rhythmic delta activity, ictal and interictal epileptiform discharges, periodic discharges and EDB. Status epilepticus was defined as the occurrence of virtually continuous or repetitive epileptiform seizure pattern in an EEG, whereas seizure pattern was defined as a phenomenon consisting of repetitive epileptiform EEG discharges at >2 c/s and/or characteristic pattern with quasi-rhythmic spatio-temporal evolution (i.e. gradual change in frequency, amplitude, morphology and location).[15] EEG findings were subdivided into 4 categories: 1) Normal EEG. 2) Normal posterior rhythm (reactive, posterior dominant rhythm with an age-appropriate frequency) with diffuse or focal abnormalities. 3) Lack of normal posterior rhythm, with focal or diffuse abnormalities. 4) Severely abnormal EEG, defined as lack of normal posterior rhythm with A) severe slowing or B) periodic discharges or C) status epilepticus. (Adjusted from Amodio 1999)[16] EEGs showing status epilepticus (SE) were excluded for analysis of specific EEG characteristics.

Standard protocol approvals, registrations and patient consents

The study was approved by the Institutional Review Board of the Erasmus University Medical Center, Rotterdam. Informed consent was obtained in all patients.

Statistical analysis

Categorical data were analyzed with Fisher-Freeman-exact test. In the comparison between pediatric and adult patients, 8 EEG characteristics are evaluated. According to Bonferroni, p-values < 0.00625 are considered significant. Kaplan-Meier analyses are used to calculate follow up time, censoring deceased patients. Ordinal logistic regression was performed to analyze the association between characteristics of first EEG and final mRS. Mann-Whitney U test was performed to analyze the relation between posterior rhythm and duration of hospital stay. These analyses are exploratory. Therefore, p-values < 0.05 are considered significant, but should be interpreted with caution. SPSS Statistics 21 (IBM Corp., Armonk, NY) was used for analysis.

Figure 1

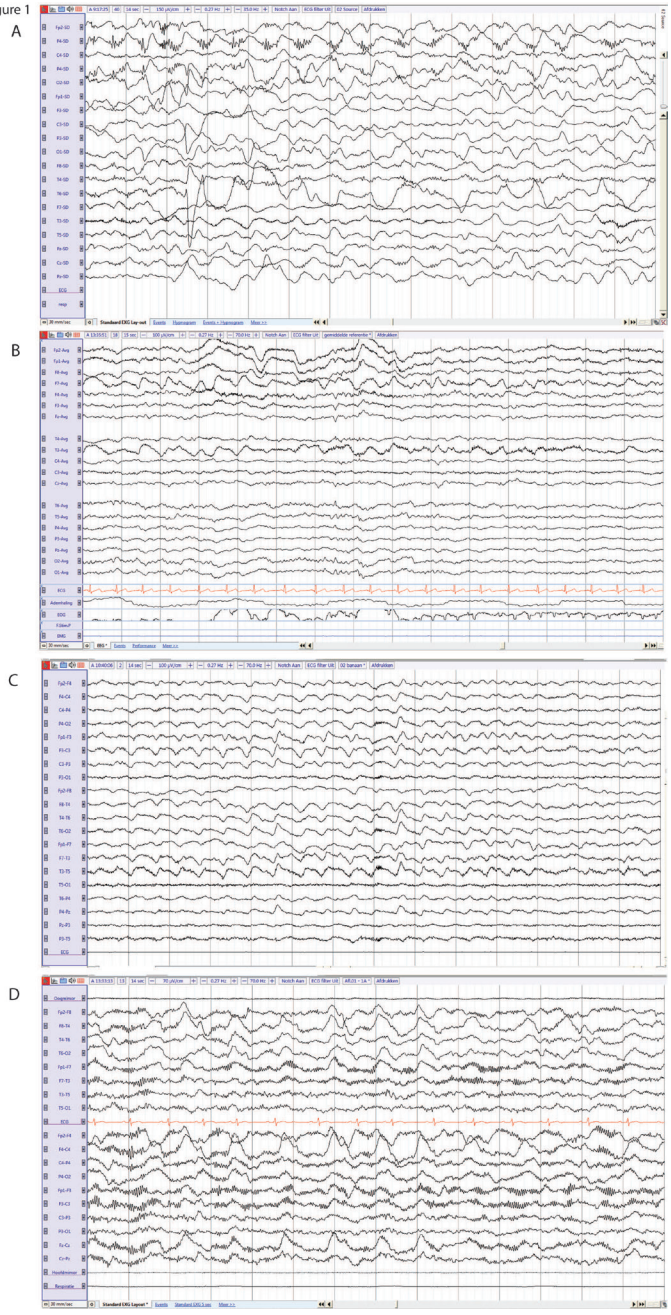

Figure 1. EEG fragments. A. Rhythmic delta activity mixed with polyspikes over the left frontal region, consistent with a focal seizure (asymptomatic). (Pre-school child, EEG in source derivation, 150 Hz/cm, high pass filter 0.27 Hz; low pass filter 35 Hz; notch filter on) B. Rhythmic delta activity at 2 Hz over the left (fronto)temporal region. (Adolescent, EEG in average reference montage, 100 Hz/cm, high pass filter 0.27 Hz; low pass filter 70 Hz; notch filter off) C. Generalized periodic discharges (Adolescent, EEG in bipolar double banana, 70 Hz/cm, high pass filter 0.27 Hz; low pass filter 70 Hz; notch filter on) D. Left frontal rhythmic delta activity at 2 Hz with superimposed burst of rhythmic 22 Hz beta frequency, consistent with the pattern of extreme delta brush (Adolescent, EEG in bipolar double banana montage, 70 Hz/cm, high pass filter 0.27 Hz; low pass filter 70 Hz; notch filter on)

Results

Patient and disease characteristics

70 adults and 35 children tested positive for NMDAR-antibodies. EEG recordings were available in 53 patients (35 adults and 18 children), all included in the study (See Fig 1 for EEG fragments). Of the adult patients, 30/35 were female and median age was 26 years (range 18-74, Table 1). Tumors were present in 10/28 female patients, including ovarian teratoma (n=8), SCLC (n=1) and merkel-cell carcinoma (n=1). All five male patients had no tumor. All but three adult patients received immunotherapy. Two untreated patients died, in both cases diagnosis was established post-mortem. Eighteen pediatric patients were included in the analyses, of which 14 were female.[17] Five children were younger than 12 years of age. Three children had an ovarian teratoma. These patients were 13 to 17 years old at disease onset. All children were treated with immunotherapy, and all pediatric patients survived.

Table 1: patient characteristics

	Adults (n = 35)		Children (n = 18)	
Female sex	30/35	(86%)	14/18	(78%)
Age at onset, median (IQR, range)	26	(21 – 48, 18 – 74)	14.5	(7 – 17, 3 – 17)
Clinical seizures	26/34	(76%)	15/18	(83%)
mRS at maximum disease severity				
- 1	-		-	
- 2	1/35	(3%)	2/18	(11%)
- 3	15/35	(43%)	8/18	(44%)
- 4	2/35	(6%)	2/18	(11%)
- 5	17/35	(49%)	6/18	(33%)
Admission to the ICU	18/35	(51%)	6/18	(33%)
First line immunotherapy	32/35	(91%)	18/18	(100%)
Second line immunotherapy	14/35	(40%)	6/18	(33%)
Follow up in months, median (IQR)	13	(9 – 18)	25	(14 – 46)
mRS at follow up (> 6 months)				
- 0	5/35	(14%)	5/17	(29%)
- 1	13/35	(37%)	4/17	(24%)
- 2	9/35	(26%)	6/17	(35%)
- 3	2/35	(6%)	-	
- 4	1/35	(3%)	2/17	(12%)
- 5	-		-	
- 6	5/35	(14%)	-	

Adult patients: cross-sectional EEG analysis

Median time from disease onset to first EEG recording was 19 days (Table 2). At the timing of first EEG, only 3/34 patients were admitted to the ICU. Two-third of the patients had normal posterior rhythm, often with focal (65%) or diffuse (65%) slowing. Diffuse slowing was either mild (n = 9), moderate (n = 6) or severe (n = 7). Epileptic discharges were present in 24% of the recordings. Two patients had EDB on their first recording. Their functional scores were mRS 4 and 5 during EEG, and one of them was

admitted to the ICU. Two patients had a normal EEG; no follow-up EEG was done, and both patients had a favorable outcome (mRS 0 and 1).

17 patients had follow up EEG at maximum disease severity. Ten patients were admitted to the ICU. 2 patients (12%) had status epilepticus. 14/15 (93%) had diffuse slowing. EDB were present in 3/15 patients. They had a mRS of 4 (n = 2) or 5 (n = 1), and one of them was in the ICU at that time. 6/15 (40%) patients still had normal posterior rhythm.

Table 2: cross-sectional EEG results

	Adults (n = 35)		**Children (n = 18)**		**p-value**
First EEG					
Time to first EEG in days, median (range)	19	(0 – 125)	8	(1 – 105)	0.61
Status epilepticus	1/35	(3%)	0/17	(0%)	1.00
EEG patterns					
- Normal posterior rhythm	21/33	(64%)	14/18 (78%)		0.53
- Diffuse slowing	22/34	(65%)	13/18 (72%)		0.76
- Focal slowing	22/34	(65%)	16/18 (89%)		0.10
- Rhythmic delta activity	14/34	(41%, 7 FIRDA, 7 TIRDA)	14/18 (78%, 4 FIRDA, 8 TIRDA, 2 OIRDA)		0.02
- Interictal epileptic discharges	6/34	(18%)	3/18 (17%)		1.00
Normal EEG	2/35 (6%)		0/18 (0%)		0.54
Follow up EEG (disease nadir)*					
Status epilepticus	2/17 (12%)		0/9 (0%)		0.53
EEG patterns					
- Normal posterior rhythm reactivity	7/15	(47%) / 6/7 (86%)	4/9	(44%) / 3/4 (75%)	1.00
- Diffuse slowing	14/15	(93%)	8/9	(89%)	1.00
- Focal slowing	7/15	(47%)	8/9	(89%)	0.08
- Rhythmic delta activity	7/15	(47%, 4x FIRDA, 2x TIRDA, 1x OIRDA)	7/9	(78%, 3x FIRDA, 1x TIRDA, 1x OIRDA, 2x GRDA)	0.21
- Interictal epileptic discharges	2/15	(13%)	1/9 (11%)		1.00
- Ictal epileptic discharges	2/15	(13%)	4/9 (44%)		0.15
- Periodic discharges	2/15	(13%)	4/9 (44%)		0.15
- EDB	3/15	(20%)	0/9 (0%)		0.27
Normal EEG	0/17		0/9		1.00

P-values < 0.00625 are considered significant (Bonferroni). *Median time between first EEG and follow up EEG was 21 days (IQR 11.5-36). TIRDA = temporal intermittent rhythmic delta activity, FIRDA = frontal intermittent rhythmic delta activity, OIRDA = occipital intermittent rhythmic delta activity, GRDA = generalized rhythmic delta activity.

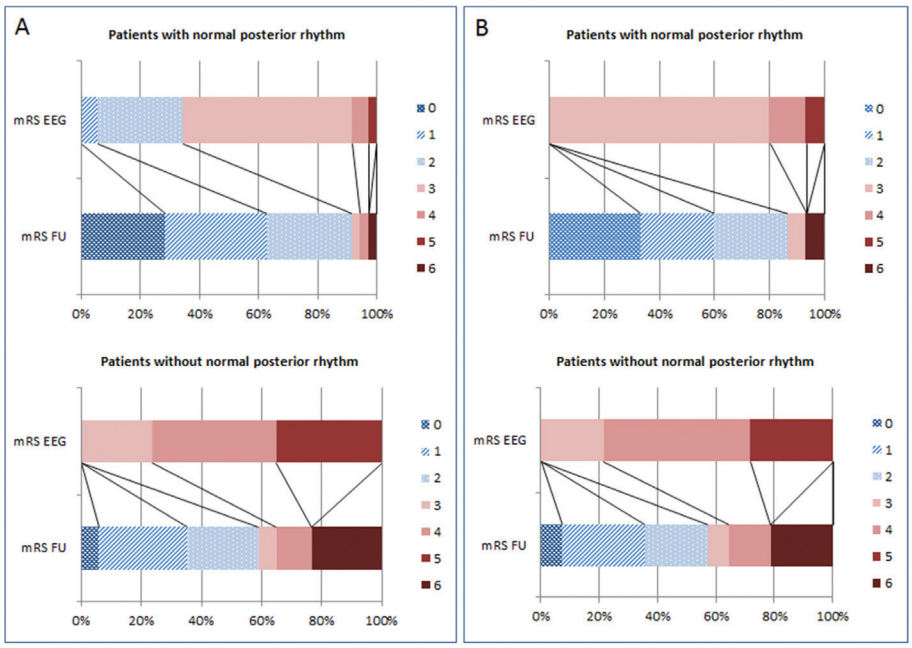

Figure 2. Predictive value of a normal posterior rhythm on first EEG recordings. Figure shows functional status measured with mRS during first EEG, and at final follow up, comparing patients with and without normal posterior rhythm. A) The unselected population (n = 52), B) The subgroup of patients with severe disease (mRS≥ 3) and early EEG (within 30 days) (n = 29). mRS = modified Rankin Scale. FU = follow up.

Pediatric patients: cross-sectional EEG analysis

Median time from disease onset to first EEG recording was 8 days. (Table 2) There were no normal EEGs. However, 14/18 patients had normal posterior rhythm. Focal slowing (89%) and diffuse slowing (72%) were common. One child had EDB on his first EEG (mRS = 5), he was the only pediatric patient admitted to the ICU during first recording.

Nine pediatric patients had follow up EEG at maximum disease severity, of which three were admitted to the ICU. 3/9 (33%) children still had normal posterior rhythm. Focal slowing (89%), diffuse slowing (89%) and intermittent rhythmic delta activity (78%) were common.

There was a trend towards more rhythmic delta activity in pediatric EEGs compared to adult patients, but differences between the populations were not significant (Table 2).

Predictive value of first EEG recording

The predictive value of normal posterior rhythm on first EEG recording could be analyzed in 52 patients (Figure 2 and 3A). 35 patients had normal posterior rhythm, of which 32 patients had a favorable outcome (91%). 17 patients had no normal posterior rhythm, of which only 10 patients had a favorable outcome (59%). Ordinal logistic

regression shows that the presence of a normal posterior rhythm was associated with lower mRS at final follow up (OR 4.74; 95% CI 1.56 – 14.47; p = 0.006). To explore whether limited disease was a confounding variable for both good EEG result and good clinical outcome, we restricted the analyses to the 40 patients with mRS ≥ 3 during first recording. The association between normal posterior rhythm and better clinical outcome remained (OR 4.28; 95% CI 1.29 – 14.25; p = 0.018). To mimic the clinical setting, we further narrowed the analyses to those with mRS ≥ 3 during first EEG within 30 days after onset. With these test limitations, 29 patients could be included. 15 patients had normal posterior rhythm, of which 13 patients had a favorable outcome (87%). 14 patients had no normal posterior rhythm, of which 8 patients had a favorable outcome (57%). Time since disease onset was not significantly different between patients with and without normal posterior rhythm. Although the frequencies of good outcome were similar, the association was no longer significant (OR 3.95, 95% CI 1.00-15.64; p = 0.051), due to smaller sample size.

The presence of normal posterior rhythm on first EEG was also associated with shorter hospital stay, which probably reflects early recovery. Patients who had died during hospital stay were excluded. Data were available in 45 patients. Thirty-two patients had a normal posterior rhythm with a median hospital stay of 35.5 days (range 0-338). Thirteen patients did not have a normal posterior rhythm, and their median hospital stay was 67 days (range 31-551, p = 0.003). Looking from the other perspective, a severely abnormal first EEG was associated with higher final mRS (OR 0.23; 95% CI 0.07 – 0.74; p = 0.014). However, more interestingly, 8/14 patients with a severely abnormal first EEG still had a favorable outcome in the end.

Longitudinal EEG analysis

EEG recordings from 13 patients (6 children and 7 adults) were available for inclusion in the longitudinal analysis (Figure 3B). Initially, eight patients had a normal posterior rhythm with focal or diffuse abnormalities (category 2). EEG worsened (to category 4) in three patients. EEG remained only slightly abnormal in the other five patients, also including patients with severe disease (mRS 4 or 5). Three patients had a severely abnormal EEG at week 1-2. Their EEG recordings had not improved at one and three months into disease. However, one of these patients finally improved well (mRS = 1). All patients with a severely affected EEG during the course of disease had a mRS of 5 at nadir.

Discussion

We report an extensive analysis including systemic re-evaluation of EEG data in over fifty patients with anti-NMDARE. Most relevant findings are 1) first EEG recordings have a predictive value for clinical outcome, 2) diffuse and focal slowing are the most

common EEG findings, 3) normal EEG does not exclude anti-NMDARE, 4) EDBs are only present in severely affected patients but not necessarily admitted to the ICU, 5) long-term severe electrographic abnormalities can be followed by good clinical outcome. Electrographic abnormalities in the pediatric and adult population were comparable.

The first EEG recording has a predictive value for final clinical outcome. The relation between mild disease, normal posterior rhythm and better outcome has been reported earlier in a study of 9 pediatric cases.[4] In our large unselected cohort, we have shown that a normal posterior rhythm on first recording predicts a favorable clinical outcome, while a severely abnormal EEG is associated with poor outcome. To analyze whether the EEG adds information to the clinical findings, we restricted our subsequent analyses to patients with mRS ≥ 3. The association between normal posterior rhythm and better clinical outcome remained, showing that normal posterior rhythm also in patients with clinically severe disease predicts a better outcome. Only a trend towards significance (p=0.051) is found if analysis is restricted to patients with mRS ≥ 3 during first EEG recorded within 30 days after onset. This is probably due to the limited number of patients in the latter analysis, as the differences between groups (frequencies of good outcome and OR) remained similar.

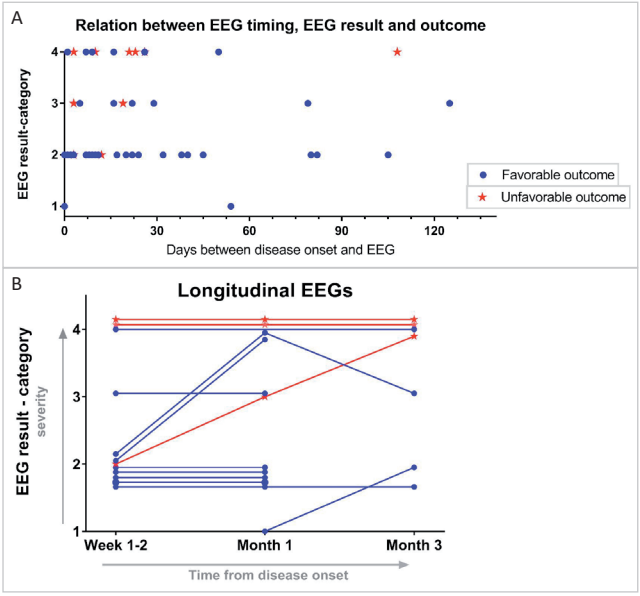

Figure 3: A) Relation between timing of first EEG, EEG category and final clinical outcome in 52 patients. B) Longitudinal EEG analyses in 13 patients. Patients with favorable clinical outcome are marked in blue (dots), patients with unfavorable outcome are marked red (stars). EEG categories: 1: normal EEG. 2: normal posterior rhythm with diffuse or focal abnormalities. 3: lack of normal posterior rhythm, with focal or diffuse abnormalities. 4: severely abnormal EEG (lack of normal posterior rhythm with severe slowing or periodic discharges or status epilepticus)

EEG is abnormal in the vast majority of the anti-NMDARE patients, but we have shown that a normal EEG registration does not exclude the diagnosis. Unremarkable EEG was seen in 4% of our patients, compared to 0-10% in earlier reports.[1-3,7,18,19] In our study, EEG has a sensitivity for anti-NMDARE of 96%; which is higher than the sensitivity of MRI brain (33%) or serum antibody analysis (87%).[1,13]

Schmitt et al. were the first to report the pattern of EDB in anti-NMDARE in 2012.[10] Their study analyzed patients in a tertiary neuro ICU with continuous EEG registrations and found EDB in 7/23 (30%) patients. Since then, several studies described EDB in subgroups: EDB were present in 9/17 (53%) pediatric patients and in 16% of children and adult patients at peak stage of disease.[3,20] A meta-analyses in pediatric anti-NMDARE calculated incidence of EDB as 16%.[21] In our unselected group, EDB were present in only 6/53 (11%) patients, either at first EEG registration (n=3) or only at follow up EEG (n=3). All six patients had mRS ≥ 4 when EDB were present, three patients were admitted to the ICU. The estimated overall incidence of EDB in anti-NMDARE is 10-15%, and this unique pattern only occurs during severe illness.

Longitudinal analyses of EEG recordings showed that EEG in the course of the first month remained stable or worsened. Lack of improvement of EEG is consistent with the earlier clinical observation that anti-NMDARE progresses over the first weeks of disease.[1] We did not identify EEG patterns related to specific stages of disease, as reported earlier in five pediatric cases.[22] Severely abnormal EEG was only seen in patients with severe clinical disease, while slightly abnormal EEGs were present in patients with either mild or severe disease. Long-term severe electrographic abnormalities can be followed by good clinical outcome, which is important in clinical decision making.

Due to the retrospective nature of our study, we were not able to collect EEG registrations on pre-defined stages of disease. Therefore, the availability of follow up EEGs was likely subject to selection bias. We analyzed the EEGs from begin stage and during maximum disease severity (if available) to obtain clinically most relevant data. In addition, the retrospective design made it impossible to perform structured cognitive assessment during disease. The advantage of the retrospective study design is the opportunity to include over fifty patients. Most reliable data were obtained by independent re-evaluation of all registrations by two experienced neurophysiologists.

We have shown that the sensitivity of an abnormal EEG is high, but normal EEG does not exclude the diagnosis anti-NMDAR encephalitis. EDBs are only present in severely affected patients. Most importantly, the first EEG recording has a predictive value for clinical outcome. A normal posterior rhythm on first recording predicts a favorable clinical outcome, while a severely abnormal EEG is associated with poor outcome.

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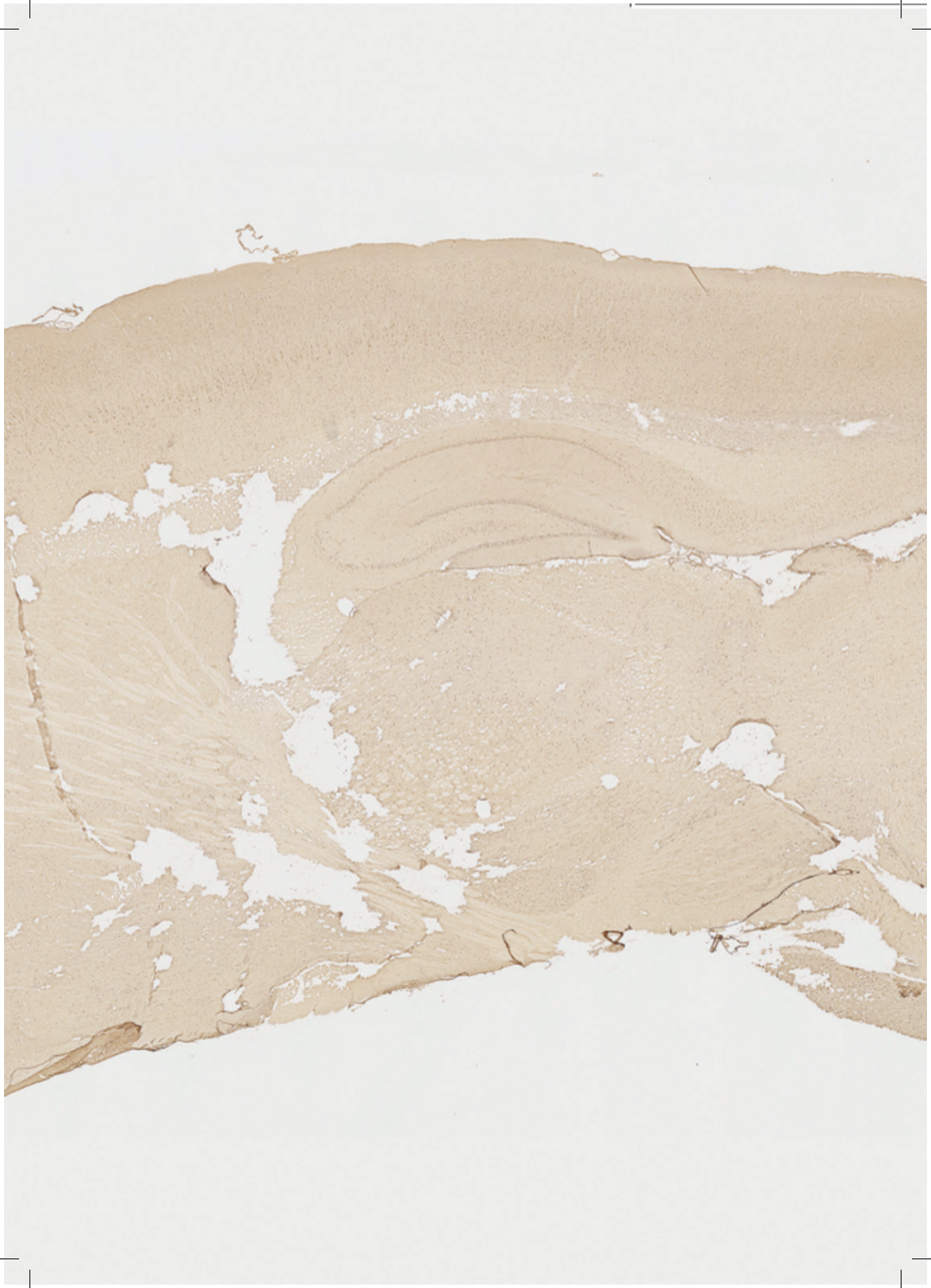
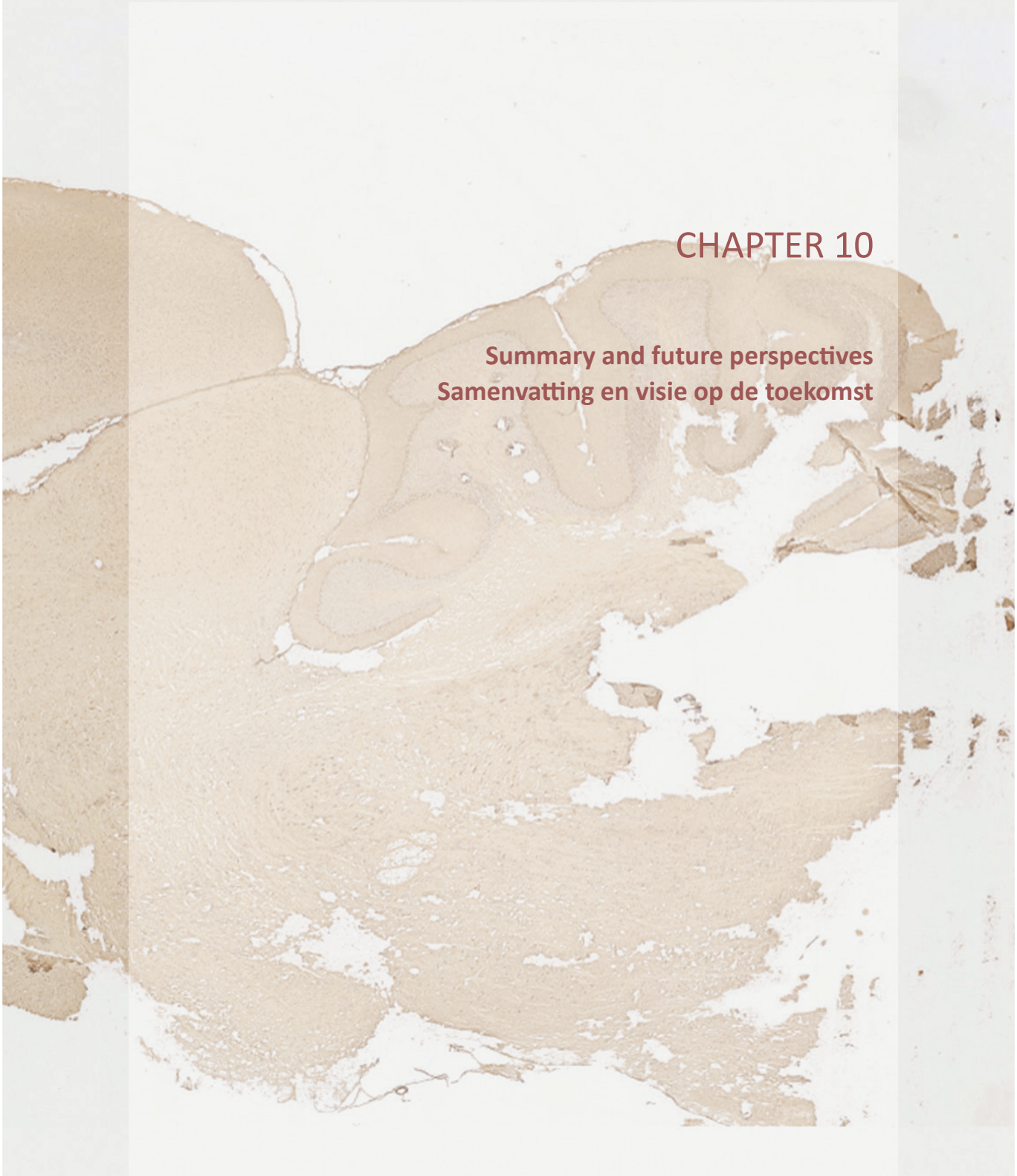




CHAPTER 10

Summary and future perspectives
Samenvatting en visie op de toekomst

Summary

Several antibodies to extracellular antigens have been discovered in the last ten years. The diseases are very interesting, not in the least because prompt recognition may lead to successful treatment. This thesis aimed to gain more insight in the most prevalent diseases associated with antibodies to extracellular antigen.

Part I: The presynaptic VGKC-complex

The voltage-gated potassium channels (VGKC) are present on the membrane of neurons in both the central and peripheral nervous system, where these contribute to depolarization following action potentials. Initially, disease was thought to result from antibodies to the VGKC itself, but attempts to show reactivity to VGKC-transfected cells failed. This had led to the discovery that antibodies were not directed to the VGKC itself, but to associated proteins: leucine-rich glioma-inactivated 1 (LGI1) and contactin-associated protein-like 2 (Caspr2).

LGI1 is a synaptic protein, mainly expressed in the hippocampus and temporal cortex. Antibodies to LGI1 result in a typical limbic encephalitis, evolving in weeks to months (Figure 1). Two-third of the patients are male and median age at onset is 64 years. Our study reports the first incidence rates and shows a dramatic increase over recent years: the annual incidence in the Netherlands was 0.83/million in 2015, compared to 0.22/million in the three years before. Increase of incidence is probably due to better recognition. More insight in the clinical syndrome will further improve recognition in the future. Patients usually have disorders of memory, behavior and spatial orientation. The vast majority of the patients have one or more seizures types. Early in disease, patients have frequent faciobrachial dystonic seizures or subtle focal seizures. Especially subtle focal seizures are easily missed, while they are a clue for early diagnosis, and this seizure type had not been clearly described in anti-LGI1 encephalitis before. Recognizing these seizures is essential for early diagnosis and treatment, preventing further cognitive impairment. Tonic clonic seizures usually occur at maximum disease severity. 65% of the patients have hyponatremia. The majority of the patients have signs of limbic encephalitis on MRI brain. Interestingly, mesotemporal sclerosis is seen in some patients at follow up imaging. Diagnosis is based on the detection of antibodies in serum or cerebrospinal fluid (CSF). However, the sensitivity of commercial CSF analysis is disappointing. Our retrospective study reports effect of first line immunotherapy in 80% of the patients, with early decrease of seizures. Cognitive improvement tends to take months to a year. After follow-up over two years, two-third of the patients had a favorable outcome (mRS 0-2). 86% suffered from persistent amnesia for the disease period, which was often very stressful to them and their relatives. It is often experienced as sign of ongoing disease, while it should be considered a sequela. Better patient counseling will be valuable. Neuropsychological assessment showed residual impairment

of spatial recognition. 35% of the patients had a relapse, although this could be an overestimation as some patients were only diagnosed at relapse. **(Chapter 2)**

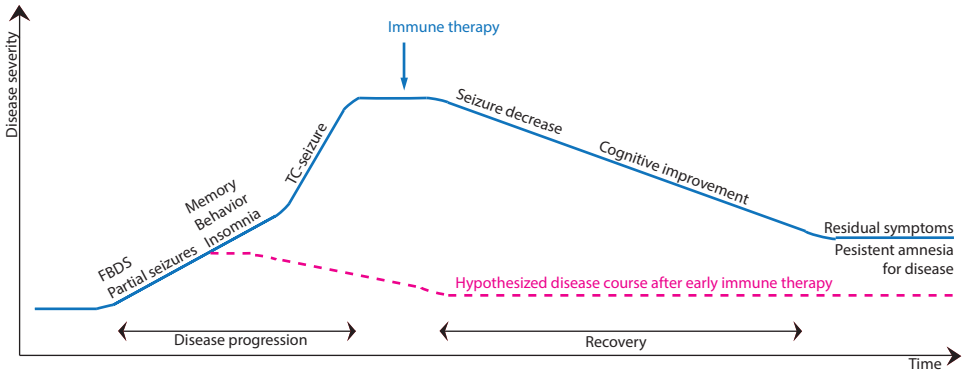


Figure 1: Disease course in anti-LGI1 encephalitis. The continuous blue line is based on our study results. In the future, we expect that earlier recognition of disease with prompt start of treatment will limit disease severity and improve final outcome. This hypothesized disease course is marked with the dashed pink line.

Antibodies to LGI1 are thought to be pathogenic, although this has not been proven by passive transfer yet, as required according to the Witebsky postulates. Some indirect evidence is available. First, anti-LGI1 encephalitis shows resemblance to the genetic disruption of the LGI1 protein. The latter is known to cause epileptic syndromes in animals and in human. Second, response to immunotherapy is mostly favorable. A third support for the autoimmune hypothesis is the discovery of an HLA-association. We detected a remarkably strong association with HLA-DR7 and HLA-DRB4 in our non-tumor LGI1 patients. This is the first report of a genetic predisposition for antibody-mediated limbic encephalitis, and our findings were confirmed by a simultaneous publication from South-Korea (back-to-back publications). These results enable further studies, including the possibility to culture disease-specific T-cells from patient's peripheral blood and to analyze the target epitope of the LGI1-protein. The strong HLA association seems not to apply to the small group of four tumor patients, suggesting two different immunopathogenic pathways. Considering the small size, absence of HLA-DR7 or HLA-DRB4 in anti-LGI1 patients could raise tumor suspicion. **(Chapter 3)**

Caspr2 is a transmembrane protein in the central and peripheral nervous system. Caspr2 binds the Kv1 subtype VGKC, which is essential for clustering of VGKCs at the juxtaparanodes of myelinated axons. Antibodies are almost exclusively seen in older male patients (89%). Data regarding the clinical syndrome were very limited, as only a few dozen of patients were reported before we started our analysis of 38 anti-Caspr2 patients. Our results show that the disease can progress relatively slow, in contrast to the subacute onset of most antibody-associated encephalitis. This can lead to diagnostic delays or to misdiagnosing the disorder as a primarily neurodegenerative disease, preventing

the early use of immunotherapy. While LGI1-antibodies cause a homogenous clinical syndrome, Caspr2-antibodies are related to a variety of clinical syndromes, including limbic encephalitis, neuromyotonia and Morvan's syndrome. However, a repertoire of seven symptoms comprises the spectrum of clinical manifestations: cerebral symptoms (cognition, epilepsy), cerebellar symptoms, peripheral nerve hyperexcitability, autonomic dysfunction, insomnia, neuropathic pain and weight loss. 77% of the patients had three or more of these core symptoms, which reflect the involvement of multiple areas of the nervous system. A tumor was present in 19% of the patients, mostly thymoma. Morvan's syndrome and neuromyotonia were more common in tumor patients compared to the non-tumor group. 90% of the patients was reported to respond to immunotherapy in our retrospective analysis. After median follow up of three years, 73% of the patients had a favorable follow up (mRS 0-2). 25% of the patients had a relapse. Interestingly, relapses can involve different parts of the nervous system than the initial disease episode. **(Chapter 4)**

About half of the VGKC-positive patients have antibodies to LGI1 or Caspr2, with a well-defined clinical syndrome. The clinical presentation of VGKC-positive patients lacking antibodies to LGI1 and Caspr2 is diverse, including REM sleep behavior disorder, multiple system atrophy, peripheral neuropathy, vasculitis, non-epileptic seizures seizures and psychogenic non-epileptic seizures. This raised the question whether VGKC-positivity in the absence of antibodies to LGI1 and Caspr2 is clinically relevant. To clarify this issue, we compared VGKC-positive patients without antibodies to LGI1 or Caspr2 to VGKC-negative patients, matched by age, gender and clinical syndrome. All cases were blindly analyzed for evidence for autoimmune inflammation, according to predefined criteria. None of the criteria for autoimmune inflammation differed between the two groups. Probability of autoimmune inflammation was independent of VGKC result. However, it was strongly associated with clinical syndrome: 57% of the patients with limbic encephalitis had evidence for autoimmune disease compared to 5% of the patients with other clinical syndromes ($p < 0.01$). No cut-off value for the VGKC titer was appropriate to discriminate between patients with and without autoimmune inflammation. In conclusion, VGKC positivity (by itself) does not contribute in clinical practice. **(Chapter 5)**

Chapter six reviews the clinical syndromes of antibodies to LGI1 and Caspr2, and aims to improve recognition of the diseases. Pediatric cases are described as well. Cohort of hundreds of children have been screened, identifying only a few positive cases. Positive test results in children need to be confirmed with additional laboratory tests, because false positive (Caspr2) results are not uncommon. Chapter six also clarifies the issues regarding VGKC-positive patients without antibodies to LGI1 and Caspr2 by providing a review of the literature and the results of our analysis. **(Chapter 6)**

Part II: The postsynaptic glutamate receptors

The alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is a postsynaptic ionotropic receptor that mediates fast excitatory synaptic transmission. The receptor belongs to the family of glutamate receptors and consists of a GluA1 and GluA2 subunit. Antibody binding to one or both of these subunits results in internalization of the receptor. The initial description of anti-AMPAR-encephalitis was published in 2009 and included ten patients. We reported the clinical syndrome and outcome in an additional 22 patients. Two-third of the patients was female and median age at onset was 62 years. Most patients presented with a classical limbic encephalitis or diffuse encephalitis. Seizures and psychiatric manifestations were relatively common. Fourteen patients had a tumor, mostly small cell lung cancer or thymoma. Seven patients had additional neuronal antibodies, such as antibodies to CRMP5 or SOX1, of which six had a tumor. Their clinical syndrome and outcome were more characteristic for the additional antibody than for anti-AMPAR encephalitis. Response to immunotherapy was limited, with good response in 25% and partial response in 50%. The presence of an additional paraneoplastic antibody was the main prognostic factor for poor outcome. These findings underline the need for tumor screening and additional antibody analysis in patients with AMPAR-antibodies. **(Chapter 7)**

Anti-N-methyl-D-aspartate receptor encephalitis (anti-NMDARE) is the most common antibody-mediated encephalitis. Disease mostly affects women of childbearing age, or children. 38% of the patients have a tumor, mainly ovarian teratoma. Patients subacutely develop psychiatric symptoms, memory loss, movement disorders and seizures, often followed by intensive care unit (ICU) admission due to consciousness decline, autonomic dysfunction or hypoventilation. Routine ancillary testing, including MRI brain and CSF analysis, is needed to exclude other diagnosis. However, results have limited contribution in the detection of anti-NMDAR encephalitis. In contrast, EEG is abnormal in almost all patients, and a specific pattern called 'extreme delta brushes' has been reported. Most studies into anti-NMDAR encephalitis only briefly report EEG results, therefore data are limited. We re-evaluated EEG registrations in 53 pediatric and adult patients, and analyzed the predictive value of first EEG. We have shown that the presence of physiological reactive background activity on first EEG registration is associated with better clinical outcome. Severely abnormal first EEG was associated with worse outcome, but this should be interpreted with caution as 8/14 patients with a severely abnormal EEG finally had a good outcome. **(Chapter 9)**

Most patients show good response to immunotherapy. Over fifty percent of the patients respond to first line therapy within four weeks. If needed, second line therapy with rituximab and cyclophosphamide is started. Overall, 81% of the patients have a favorable outcome after two years. Unfortunately, these good results on group level do not apply to every individual patient. We have reported a severe case of anti-NMDAR encephalitis in a 25-year old woman. She was admitted to the ICU for almost a year.

NMDAR-antibody titer remained high, despite removal of bilateral ovarian teratoma and extensive first and second line immunotherapy. This is the first report of intrathecal administration of rituximab in anti-NMDAR encephalitis. It was safely used but extensive laboratory results did not support a direct effect on the central nervous system. After 1.5 years of hospital stay, patient finally improved. This severe case underlines the need for perseverance in treating anti-NMDAR encephalitis. **(Chapter 8)**

Future perspectives

In the last decade, there have been major breakthroughs in the field of antibody mediated encephalitis and great developments will follow in coming years. Patient numbers will increase due to better recognition, and the detection of new antibodies is expected. However, research should be planned thoughtfully. We should be vigilant for misinterpretation (or overinterpretation) of laboratory results in this rapidly developing scientific field. With careful patient selection, a HLA link might be detected in patients with antibodies to NMDAR or Caspr2, just as in anti-LGI1 encephalitis. Other interesting steps forward will be the development of predictive models for (long-term) outcome, and the development of evidence-based treatment strategies.

Research strategies

Research is complicated by the rare occurrence of specific antibodies and therefore it seems attractive to lump patients with different antibodies. However, this should be avoided as antibodies and their syndromes differ significantly. Extrapolating data from well-studied antibodies (i.e. NMDAR) to rare antibodies is probably not very reliable. Antibodies differ in their pathophysiological characteristics, including different IgG subtypes (IgG1 NMDAR antibodies vs IgG4 LGI1 and Caspr2 antibodies) and different sites of antibody production (intrathecal vs systemic). Also, epidemiology differs per antigen, including age at onset (anti-NMDARE in children and adolescents vs anti-Caspr2 encephalitis in the elderly), and gender (female anti-NMDARE patients compared to male anti-Caspr2 patients) and there are relevant differences in clinical syndromes.

Clinicians and laboratory experts need to collaborate to avoid misinterpretation of laboratory results. A clear example is the positive VGKC-radioimmunoassay, which has resulted in diagnosis of 'anti-VGKC-encephalitis' for years. The unlimited clinical presentations of VGKC-positivity should have alarmed more physicians and researchers. This was ultimately proven in 2010 with the discovery of the clearly distinct subgroups of anti-LGI1 and anti-Caspr2 encephalitis patients, and later with our description of the lack of clinical significance of VGKC-positivity in the absence of these two antibodies. This misinterpretation of VGKC-results should be a warning. But in contrast, some laboratories still report VGKC-patients without providing results of LGI1 and Caspr2 tests, and reviewers still allow this. In addition, Caspr2 and NMDAR serum cell-based

assays (CBA) have been shown to be positive but clinically irrelevant in some patients, and need confirmation with CSF, or with immunohistochemistry or cell cultures in all patients. However, some study groups have included these patients in their studies without laboratory confirmation. These studies blur research, create the false impression that the clinical phenotype expands, and reinforce the confusion about relevant or irrelevant antibodies.

HLA-associations

The discovery of an HLA-association in non-tumor anti-LGI1 patients creates curiosity about a possible HLA-link in patients with other antibodies. As a pilot, we have performed HLA analysis in ten anti-NMDARE patients without a teratoma, but we were not able to detect an association yet, neither did a Korean study group in 17 patients. Confirmation of both the HLA link in anti-LGI1 encephalitis and the lack of an HLA association in a large group of anti-NMDARE patients was also found and will soon be published by a German study group. An HLA-link in anti-NMDARE patients might be truly absent. But possibly, a HLA-link is present but currently not observed in unselected patient groups. Anti-NMDARE is a more heterogeneous disease (compared to anti-LGI1 encephalitis), with a heterogeneous epidemiology: pediatric patients vs elderly, with or without teratoma, postviral or idiopathic. This might be disturbing the evaluation of HLA results. In contrast, HLA analyses have been more straightforward in the homogeneous group of anti-LGI1 patients, all presenting with similar disease courses. Careful clinical observation including ancillary testing of anti-NMDARE patients (truly translational research) will possibly enable the selection of a homogenous subgroup in which a HLA-association might be detected. As far as we know, HLA analyses have not been performed in patients with anti-Caspr2 disease yet. Results in this interesting subgroup are difficult to predict, as this patient group is remarkably homogenous, but with very diverse clinical phenotypes.

Outcome prediction

The development of predictive models for clinical outcome is useful for several reasons: to provide information for patients and relatives, to assess the need for extensive first- or second line pulse therapy in the acute phase and for maintenance therapy in the long-term, and to offer appropriate (cognitive) rehabilitation programs. In addition, a predictive model allows for the selection of patients for therapy trials. Predictive models will differ per disease and antibody, and the development is complicated by the large number of patients needed to analyze. Several outcome predictors are available for the most common autoimmune encephalitis (anti-NMDARE), and should be included in a predictive model. Early start of immunotherapy, no need for ICU admission and lower CSF antibody titers are associated with better outcome, as is normal posterior rhythm on first EEG. The course of antibody titers and CXCL13 levels in CSF seem

to be predictive as well, but these data are not available early into disease. Brain FDG/PET hypometabolism in the occipital lobes correlates with disease severity, and might be predictive of outcome, but it is not widely available and therefore not a useful marker. Gender, age and tumor presence were not distinctive in earlier studies.

Evidence-based treatment

Therapy studies will soon be feasible, as patient numbers increase due to better recognition and international collaboration continues. Patients with the most common antibodies, directed to NMDAR and LGI1, form the best groups for initial studies. The three most relevant and feasible research questions are: 1) what is the best first-line treatment option, comparing methylprednisolone, intravenous immunoglobulins, plasma exchange or a combination, 2) what is the best second-line treatment option, and when should it be initiated, and 3) is maintenance therapy effective in preventing relapses, and which steroid-sparing option is safe and most effective? The second question mainly refers to anti-LGI1 encephalitis. Preferentially, a double-blind randomized controlled trial is initiated, but such a trial across borders in these rare diseases will probably be very complex. A good alternative will be a study design in which different sites follow their own predefined protocol. Inclusion and exclusion criteria should be similar, and the same clinical and laboratory measurements should be obtained on predefined stages of disease. Outcome measures need to be disease-specific and sensitive. For example, long term cognitive deficit is common in anti-LGI1 encephalitis, but will be insufficiently clarified with the rough modified Rankin Scale. A good option to assess cognitive outcome, independent of language and culture, would be a computerized neuropsychological assessment battery, for example CANTAB (see chapter 2). Follow up should be at least one year. However, a study arm with extended follow up of three years would be very interesting to analyze the effects of maintenance therapy.

In conclusion, exciting years are to come in the rapidly developing field of autoimmune encephalitis. Interesting questions will hopefully be answered with transparent research. The strong link between clinicians and laboratory experts is essential. New laboratory findings should lead to extensive clinical analyses of the study group and controls. In addition, selection of subgroups of patients with similar clinical characteristics is probably the most promising starting point for the discovery of new antibodies. Further expansion of international collaboration is useful to increase patient numbers in research projects, which also allows for therapy trials. With these efforts, early reliable diagnoses and evidence based treatment strategies will come within reach.

Samenvatting

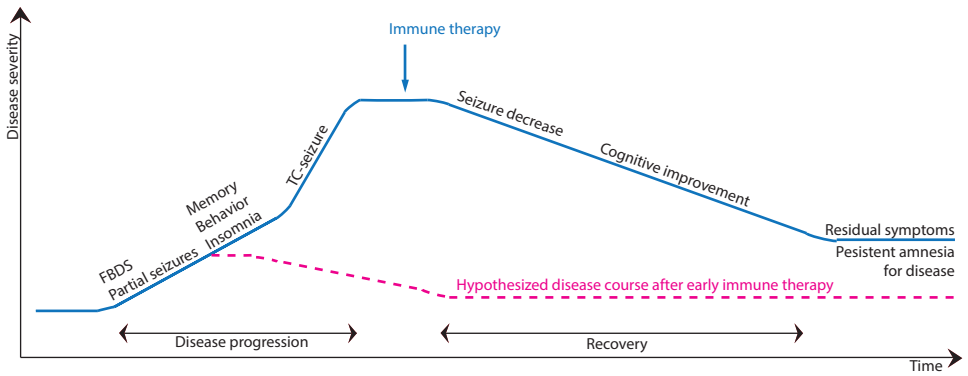
Sinds 2007 zijn er verschillende antistoffen ontdekt die zich richten tegen extracellulaire antigenen en een neurologische ziekte veroorzaken. Vlotte herkenning van de ziekte is essentieel, omdat de aandoeningen veelal behandelbaar zijn. Het doel van dit proefschrift is om meer inzicht te geven in de meest voorkomende ziekten die geassocieerd zijn met antistoffen tegen extracellulaire antigenen.

Deel 1: Het pre-synaptische VGKC-complex (kaliumkanaal)

Het spanningsafhankelijke kalium kanaal (VGKC) is aanwezig op neuronen in het centrale en perifere zenuwstelsel. Het kanaal zorgt ervoor dat de cel na een actiepotentiaal terugkeert naar de rusttoestand. Aanvankelijk werd gedacht dat antistoffen zich direct tegen het kaliumkanaal richtten, maar in het laboratorium werd dit niet aangetoond. In 2010 werd ontdekt dat antistoffen zich richten tegen twee eiwitten die een nauwe relatie hebben tot het kaliumkanaal: LGI1 en Caspr2.

LGI1 is een eiwit dat wordt uitgescheiden in de synaps tussen twee zenuwcellen. De hersengebieden waar veel LGI1 aanwezig is, zijn de hippocampus en de temporaalschors. Antistoffen tegen LGI1 veroorzaken een hersenontsteking genaamd limbische encefalitis. Patiënten ontwikkelen in enkele weken tot maanden geheugenklachten, gedragsveranderingen en epilepsie. Twee derde van de patiënten is man en de mediane leeftijd is 64 jaar. Uit ons onderzoek komen de eerste incidentiecijfers naar voren. De jaarlijkse incidentie in Nederland was 0.83/miljoen in 2015, vergeleken met 0.22/miljoen in de drie jaren daarvoor. Deze opvallende stijging is waarschijnlijk veroorzaakt door betere herkenning van de ziekte en meer inzicht in de ziekte zal de incidentiecijfers verder doen toenemen. Patiënten hebben verschillende vormen van epilepsie. Vroeg in de ziekte komen specifieke faciobrachiale dystone aanvallen voor, evenals subtiele focale insulten. Met name de focale insulten worden veel gemist, terwijl deze een clou zijn tot het stellen van de diagnose. Dit is essentieel, omdat snelle behandeling (verdere) achteruitgang van de cognitie kan voorkomen. Tonisch clonische epileptische insulten komen vooral voor als de ziekte op zijn maximum is. Een verlaagd natrium in serum (bloed) komt voor bij 65% van de patiënten. De meerderheid van de patiënten heeft tekenen van een limbische encefalitis op de MRI van de hersenen. Op follow up scans wordt soms mesotemporaal sclerose gezien. De diagnose anti-LGI1 encefalitis is gebaseerd op het aantonen van de antistoffen in serum of liquor (hersenvocht), waarbij in onze studie serum sensitiever bleek. In onze retrospectieve studie verbeterde 80% van de patiënten op eerstelijns immuuntherapie. Epilepsie vermindert snel, terwijl cognitief herstel maanden tot een jaar duurt. Na minimaal 2 jaar follow up had twee derde van de patiënten een gunstige uitkomst (mRS 0-2). 86% van de patiënten had nog amnesie voor de ziekteperiode. Dit gaf veel zorgen bij patiënten en hun naasten die dit beschouwden als teken van voortdurende ziekte, terwijl het in werkelijkheid een restverschijnsel is.

Goede voorlichting is hierom van belang. Neuropsychologisch onderzoek toonde veelal nog een beperking van de ruimtelijke oriëntatie. Anamnestisch was ook het executief functioneren gestoord, maar dit is niet formeel getest. 35% van de patiënten had een recidief van de ziekte, echter dit kan een overschatting zijn doordat een aantal patiënten pas tijdens het recidief werd gediagnosticeerd. **(Hoofdstuk 2)**



Figuur 1: Ziektebeloop van anti-LGI1 encefalitis. The blauwe lijn is gebaseerd op resultaten van onze studie. We verwachten dat snellere herkenning en vlotte start van behandeling in de toekomst leidt tot minder ernstige ziekte en betere klinische uitkomsten. Dit veronderstelde ziektebeloop is aangegeven met de roze stippellijn.

Antistoffen tegen LGI1 worden als pathogeen beschouwd, hoewel dit nog niet door passieve overdracht is bewezen. Er is wel indirect bewijs. Allereerst lijkt de ziekte op de genetische verstoring van het LGI1 eiwit, hetgeen een epilepsie syndroom veroorzaakt. Ten tweede is immuuntherapie meestal werkzaam. Een derde ondersteunend bewijs voor de auto-immuun hypothese is de HLA-associatie die wij beschrijven in hoofdstuk 3. We hebben een opvallend sterke associatie ontdekt met HLA-DR7 en HLA-DRB4 in onze anti-LGI1 patiënten zonder tumor (88%). Dit is de eerste ontdekking van een genetische predispositie voor een antistof gemedieerde limbische encefalitis, en onze resultaten zijn bevestigd in een onderzoek uit Zuid-Korea dat gelijktijdig is gepubliceerd (back-to-back publicatie). De sterke HLA-associatie vonden we niet terug in de vier patiënten met een tumor, hetgeen mogelijk een andere pathofysiologie suggereert in deze groep. Dit moet uiteraard bevestigd worden in een grotere groep patiënten, maar afwezigheid van HLA-DR7 en HLA-DRB4 bij anti-LGI1 patiënten kan de verdenking op een tumor doen stijgen. **(Hoofdstuk 3)**

Caspr2 is een transmembraan eiwit in het centrale en perifere zenuwstelsel. Caspr2 is essentieel voor het clusteren van kaliumkanalen op de juxtaparanodale regio van gemyeliniseerde axonen. Antistoffen komen voornamelijk voor bij oudere mannen (89%). Voorheen waren er weinig gegevens over de ziekte bekend, omdat er weinig patiënten waren beschreven. Wij analyseerden 38 patiënten en bemerkten dat de ziekte

zich langzaam ontwikkelt, in tegenstelling tot het subacute beloop van de meeste antistof gemedieerde encefalitiden. Het langzame beloop kan leiden tot de misdiagnose van een neurodegeneratieve aandoening, zoals een dementie, waardoor patiënten niet de juiste behandeling krijgen. Het klinisch beeld van Caspr2-antistoffen is gevarieerd: limbische encefalitis, neuromyotonie en het syndroom van Morvan komen voor. Er is wel degelijk overlap, doordat er zeven symptomen zijn die in verschillende combinaties veel voorkomen: cerebrale symptomen (cognitie en epilepsie), cerebellaire symptomen, perifere zenuw hyperexcitabiliteit, autonome dysfunctie, insomnie, neuropathische pijn en gewichtsverlies. 77% van de patiënten had drie of meer van deze symptomen. 19% van de patiënten had een tumor, met name thymomen. Het syndroom van Morvan en neuromyotonie kwamen vaker voor bij patiënten met een tumor. In ons retrospectieve onderzoek reageerde 90% van de patiënten op immuuntherapie. Na een mediane follow up van drie jaar had 73% van de patiënten een gunstige uitkomst (mRS 0-2). 25% van de patiënten had een recidief van de ziekte, waarbij soms juist een ander deel van het zenuwstelsel betrokken was dan bij de eerste ziekte episode. **(Hoofdstuk 4)**

Ongeveer de helft van de VGKC-positieve patiënten heeft antistoffen tegen LGI1 of Caspr2, met een duidelijk omschreven klinisch beeld. Het klinisch beeld van de VGKC-positieve patiënten zonder antistoffen tegen LGI1 en Caspr2 is daarentegen heel divers, waaronder REM-slaap stoornissen, multipele systeem atrofie, perifere neuropathie, vasculitis en psychogene, non-epileptische aanvallen. Dat roept de vraag op of een positieve VGKC-test in afwezigheid van antistoffen tegen LGI1 en Caspr2 wel klinisch relevant is. Om deze vraag te beantwoorden hebben wij deze patiënten vergeleken met VGKC-negatieve patiënten, gematcht voor leeftijd, geslacht en klinisch beeld. Alle casus werden blind beoordeeld op bewijs voor een auto-immuun ontsteking, aan de hand van vooraf opgestelde criteria. De twee groepen verschilden niet van elkaar en de kans op een auto-immuun ziekte was onafhankelijk van de VGKC-uitslag. Er was geen grenswaarde voor de VGKC-test die differentieerde tussen patiënten met en zonder auto-immuun ontsteking. We concludeerden dat VGKC-positiviteit zonder antistoffen tegen LGI1 en Caspr2 niet klinisch relevant is. **(Hoofdstuk 5)**

Hoofdstuk 6 geeft een overzicht van de klinische syndromen veroorzaakt door antistoffen tegen LGI1 en Caspr2, met als doel de herkenning van de ziektes te verbeteren. Ook de casus van patiënten op de kinderleeftijd worden besproken. In verschillende onderzoeken zijn honderden kinderen gescreend, waarbij slechts enkele patiënten positief bleken. Een positieve testuitslag bij een kind moet altijd bevestigd worden met aanvullende laboratoriumtesten, omdat de ziekte bij kinderen zeer zeldzaam is en vals-positieve uitslagen van met name Caspr2-antistoffen niet zeldzaam zijn. **(Hoofdstuk 6)**

Deel II: De postsynaptische glutamaatreceptoren

De AMPA receptor is een postsynaptische ionotrope receptor die een snelle synaptische signaaloverdracht reguleert. Wanneer antistoffen aan de receptor binden worden deze inactief door internalisatie. De eerste beschrijving van anti-AMPAR encefalitis dateert uit 2009. Er werden tien patiënten beschreven. In hoofdstuk 7 rapporteren wij het klinisch beeld en de uitkomst van 22 nieuwe patiënten. Twee derde van de patiënten is vrouw, en de mediane leeftijd is 62 jaar. De meeste patiënten hebben een limbische encefalitis of een diffuse encefalitis. Epileptische insulten en psychiatrische symptomen komen veel voor. Veertien patiënten hadden een tumor, meestal een kleincellige longtumor of een thymoom. Zeven patiënten hadden een tweede neuronale antistof, tegen bijv CRMP5 of SOX1, zes van hen had een tumor. Het klinisch beeld in deze patiënten paste meer bij de tweede antistof dan bij anti-AMPAR encefalitis. Slechts 25% van alle patiënten had een goede reactie op immuuntherapie, 50% reageerde deels. De aanwezigheid van een tweede antistof was de belangrijkste voorspeller voor een slechte uitkomst. Daarom is tumorscreening en onderzoek naar additionele antistoffen van belang in patiënten met AMPAR-antistoffen. **(Hoofdstuk 7)**

Anti-NMDAR encefalitis is de meest voorkomende antistof-gemedieerde encefalitis. De ziekte komt vooral voor bij vrouwen in de vruchtbare leeftijd, en bij kinderen. 38% van de patiënten heeft een tumor, veelal een ovariumteratoom. Patiënten ontwikkelen in enkele dagen tot weken psychiatrische symptomen, geheugenverlies, bewegingsstoornissen en insulten. De meeste patiënten moeten worden opgenomen op de intensive care vanwege een verlaagd bewustzijn, autonome dysfunctie en hypoventilatie. Aanvullend onderzoek zoals MRI en liquor analyse zijn nodig om andere oorzaken uit te sluiten, maar dragen nauwelijks bij aan de diagnose anti-NMDAR encefalitis. Het EEG daarentegen kan van meerwaarde zijn, omdat het specifieke patroon van 'extreme delta brushes' alleen bij deze patiënten voorkomt. In de literatuur is verder weinig informatie beschikbaar over het EEG bij anti-NMDAR encefalitis. We hebben daarom EEGs van 53 kinderen en volwassenen herbeoordeeld en de voorspellende waarde van het EEG geanalyseerd. Onze resultaten laten zien dat een normaal achtergrondpatroon op het eerste EEG voorspellend is voor een goede uitkomst (91% vs 59%). Een ernstig afwijkend EEG was geassocieerd met een slechte uitkomst, maar dit moet voorzichtig worden geïnterpreteerd omdat 8 van de 14 patiënten met een ernstige afwijkend EEG wel een goede uitkomst had. **(Hoofdstuk 9)**

De meeste anti-NMDAR patiënten reageren goed op eerstelijns immuuntherapie, maar in een deel van de patiënten is tweedelijns behandeling met rituximab en/of cyclofosfamide nodig. 81% van de patiënten heeft een gunstige uitkomst na twee jaar. Helaas zijn deze gunstige gegevens niet van toepassing op iedere individuele patiënt. In hoofdstuk 8 beschrijven wij een casus van een 25-jarige vrouw die bijna een jaar op de intensive care was opgenomen. De antistoftiters bleven hoog, ondanks het verwijderen van ovariumteratomen beiderzijds en uitgebreide eerste en tweedelijns immuuntherapie.

Dit is de eerste publicatie van intrathecale toediening van rituximab in anti-NMDAR encefalitis. Uitgebreid laboratoriumonderzoek inclusief antistoftiters en B-cel tellingen toonde geen direct effect op het centrale zenuwstelsel. Na ruim 1.5 jaar opname in het ziekenhuis herstelde patiente redelijk. Deze ernstige casus benadrukt het belang van volhardendheid bij de behandeling van anti-NMDAR encefalitis. **(Hoofdstuk 8)**

Visie op de toekomst

De afgelopen jaren zijn er grote doorbraken geweest op het gebied van antistof-gemedieerde encefalitis en nieuwe ontwikkelingen zullen volgen. Het aantal patiënten zal stijgen door betere herkenning van de ziekte en waarschijnlijk worden er nog nieuwe antistoffen ontdekt. In dit snel ontwikkelende vakgebied moeten we waken voor misinterpretatie van laboratoriumuitslagen, waarbij nauwe samenwerkingen tussen clinici en laboratorium experts essentieel is. Het is interessant om te onderzoeken of er ook bij patiënten met antistoffen tegen NMDA of Caspr2 een HLA-associatie bestaat. Andere interessante onderzoeksrichtingen zijn het ontwikkelen van een voorspellend model voor lange termijn uitkomsten, en studies naar de meest effectieve behandelstrategieën. Internationale samenwerking is daarbij van belang. Met deze inspanningen is vlotte diagnostiek en effectieve behandeling van antistofgemedieerde encefalitis hopelijk binnen handbereik. **(Future Perspectives)**

APPENDICES

Dankwoord

Graag maak ik van de gelegenheid gebruik om mijn dankbaarheid uit te spreken voor de bijdragen die velen aan mijn proefschrift hebben geleverd. Toen ik in 2014 aan dit traject begon, was er nog geen onderzoeksgroep auto-immuun encefalitis in het Erasmus MC. Er was geen database, geen expertisecentrum met landelijke bekendheid en geen spreekuur om patiënten heen te verwijzen. Dankzij de enthousiaste medewerking van vele verwijzers in het land en de bereidheid van de patiënten om mee te werken aan onderzoek, kwam hier in korte tijd verandering in. Door de prettige samenwerking met co-auteurs in binnen- en buitenland konden we in relatief korte tijd veel goede data bijeen krijgen en analyseren. Een aantal mensen wil ik in het bijzonder bedanken.

Met stip op één: mijn co-promotor dr. M.J. Titulaer. Beste Maarten, in 2012 bracht Paul Wirtz ons in contact en via skype werkten we aan een artikel. Toen jij een jaar later naar Nederland verhuisde, mocht ik bij je langs komen om eens over promotie-onderzoek te praten. Op een terrasje in Rotterdam maakten we de eerste plannen die hebben geleid tot dit boekje. Het geduld en enthousiasme waarmee je mij toen uitlegde wat VGKC was, is altijd gebleven. Ik heb veel profijt gehad van (en ik bewonder) je tomeloze kennis over alles wat met antistoffen te maken heeft. Naast je ijzersterke inhoudelijke begeleiding heb je me wegwijs gemaakt in de wereld van wetenschappers, publicaties, database programma's en statistiek, en je was altijd bereid iets voor me op te lossen als ik in Den Haag was. Mede daardoor kon ik dit promotie-traject voltooien naast mijn opleiding. Je scherpe kritiek en snelle reactie op alles wat ik je stuurde heb ik erg gewaardeerd. Een betere begeleider had ik mij niet kunnen wensen.

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Aan de basis van dit proefschrift staan laboratoriumresultaten van vele onderzochte samples. Deze data zijn grotendeels tot stand gekomen door het goede werk van onze laboranten Esther en Mariska op het JNI. Mijn dank is groot! Ook de samenwerking met Marco Schreurs en met de laboranten op het Laboratorium Medische Immunologie was van grote waarde.

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About the author

Agnes van Sonderen is geboren op 3 februari 1986 in Zeist, Nederland. In 2004 behaalde zij haar vwo-diploma aan het Sint Laurens College te Rotterdam. Hierna begon zij aan de studie Geneeskunde in het LUMC. Vanaf haar tweede jaar combineerde zij dit met succes met een bacheloropleiding Rechtsgeleerdheid, eveneens aan de Universiteit Leiden. In 2008 startte zij met haar co-schappen, onder andere in Suriname, met als afsluiting de semi-arts stage neurologie in het Haga Ziekenhuis, Den Haag. Na het behalen van het artsexamen in 2011 startte Agnes met de opleiding tot neuroloog in het Haga Ziekenhuis, bij dr. Bas de Bruijn. Vanaf 2014 combineerde zij dit met promotie-onderzoek bij prof. dr. Peter Sillevis Smitt en dr. Maarten Titulaer in het Erasmus MC, Rotterdam. In april 2018 heeft zij haar opleiding tot neuroloog afgerond. Sindsdien is zij werkzaam als neuroloog in het Haaglanden Medisch Centrum (HMC), Den Haag. Agnes woont samen met Dirk Saal en hun dochters Juliette (2016) en Lizelot (2018) in Den Haag.

List of publications

Papers included in this thesis

Van Sonderen A, Arends S, Tavy DLJ, Bastiaansen AEM, de Bruijn MAAM, Schreurs MWJ, Sillevis Smitt PAE, Titulaer MJ. *Predictive value of electroencephalography in anti-NMDA receptor Encephalitis.* Journal of Neurology, Neurosurgery, and Psychiatry. 2018 Oct;89(10):1101-1106

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Van Sonderen A, Koppen H, De Bruijn SFTM. *Thunderclap headache.* Oxford Textbook of Headache Syndromes, Chapter 34

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Van Sonderen A , Keunen RWM, Tavy DLJ, Boezeman EHJF, Roos YB, Mess WH. *De waarde van emboliedetectie bij het voorspellen en voorkomen van cerebrale ischemie, (detection of embolic signals in the prediction and prevention of cerebral ischemia)* Tijdschr Neurol Neurochir 2011;112:255-62

PhD portfolio

	Year	ECTS
General courses		
Research Integrity Course, Erasmus University Medical Center, Rotterdam, The Netherlands	2015	0.5
BROK (Basiscursus Regelgeving Klinisch Onderzoek, NFU BROK Academie)	2015	1.5
Hospital Management Course, Academie voor Medisch Specialisten, The Hague, The Netherlands.	2016	1
Specific courses		
AIOS course (3rd year) Neuro-Immunology/ Infection, Utrecht, The Netherlands	2013	1.5
Biemond course Inflammation and Infection, Veldhoven, The Netherlands	2015	0.5
Presentations		
Autoimmune encephalitis in patients with psychiatric symptoms. Psychiatrists and Neurologists in dialogue. Amstelveen, The Netherlands	2017	1
The clinical spectrum of Caspr2 antibody mediated disease. American Academy of Neurology (AAN) Annual meeting, Vancouver, Canada	2016	1.5
The clinical relevance of VGKC-complex antibodies in the absence of LGI1 and Caspr2 antibodies. Annual scientific meeting, Haga Teaching Hospitals, The Hague, The Netherlands.	2015	1
The relevance of VGKC-complex antibodies. Annual scientific meeting of the Dutch Association of Neurology (NVN) 2014, Nunspeet, The Netherlands	2014	1
The relevance of VGKC-complex antibodies. 50th Anniversary Congress 2014, Dutch Society for Immunology (NVVI), Kaatsheuvel, The Netherlands	2014	1
(Inter)national conferences		
American Academy of Neurology Annual Meeting 2016, Vancouver, Canada (attendance, oral presentation and poster presentation)	2016	1.5
The Lancet Neurology Autoimmune Disorders Conference 2015, Barcelona, Spain (attendance and poster presentation *Clinical relevance of antibodies directed to the voltage-gated potassium channel (VGKC) complex)*	2015	1.5
Scientific retreat, Department of Neuro-oncology Erasmus MC (attendance and oral presentation)	2015	1
Joint Congress of European Neurology (EFNS/ENS) 2014, Istanbul, Turkey (attendance)	2014	1.5
Teaching		
Supervising master's thesis (1 student Neuropsychology)	2015	2
The relevance of VGKC-complex antibodies. Educational meeting, Laboratory of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands	2015	1
Overview 'the approach of autoimmune encephalitis', written for the Dutch Society for Neurology (Antistof geassocieerde encefalitis: handvatten voor diagnostiek en behandeling. Tijdschr Neurol Neurochir 2016;117(2):78-88)	2016	2
Antibody mediated limbic encephalitis. Intercity Symposium Neurology, South-east Netherlands (invited speaker, grand rounds). Sittard, The Netherlands.	2015	1
Other		
Neuroimmunology meeting (multidisciplinary patient consultation, monthly). Department of Neurology, Erasmus MC, Rotterdam, The Netherlands	2015	1
Research meeting (weekly). Laboratory of neuro-oncology, Erasmus MC, Rotterdam, The Netherlands	2015	2
Clinical consultations (outpatient clinic, and advice to treating physicians who approach us by telephone/email, daily to weekly)	2015	2
		27

List of abbreviations

ADAM	a disintegrin and metalloproteinase
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Caspr2	contactin-associated protein-like 2
CBA	cell-based assay
CSF	cerebrospinal fluid
DPPX	dipeptyl-peptidase-like protein-X
EEG	electroencephalogram
GABA	γ-aminobutyric acid
GlyR	glycine receptor
LGI1	leucine-rich glioma-inactivated 1
NMDAR	N-methyl-d-aspartate receptor
PSD	postsynaptic density protein
RAI	radioimmunoassay
VGKC	voltage-gated potassium channel