1 General Information

1.1 Identification of the study

Title: **Antibody-mediated LGI1 encephalitis: symptoms, biomarkers, and mechanisms of the chronic phase of the disease**

Protocol identification code or number: **PI23/00858**
Version and date: V4; 2/2/2024

1.2 Identification of the promoter

Josep Dalmau, as principal investigator
CELLEX P3A
c/ Casanova 143

Co-IP
Lorena Rami
Alzheimer's and other cognitive disorders unit
C/Villarroell, 170

1.3 Identification of principal investigators in our center

Josep Dalmau: IDIBAPS, Cellex 3A, Ext: 1738
Lorena Rami: Fundació Clínic. IDIBAPS. 934518240

2 Justification

The discovery of the encephalitis associated with antibodies against neuronal surface antigens initiated a new era in the study of autoimmune diseases of the central nervous system (CNS) with important implications for neurology, psychiatry and neurosciences in general (1). In contrast to CNS disorders related to antibodies against intracellular proteins, patients with autoimmune encephalitis and antibodies against neuronal surface proteins, such as the NMDA receptor (NMDAR) or LGI1 protein, may or may not have an underlying tumor, the antibodies are directed against extracellular epitopes of the antigens, and have mild to moderate brain cytotoxic T cell infiltrates with predominant pathogenic mechanisms related to B cells (2). In these cases, the antibodies reversibly alter the structure and function of the target antigens (NMDAR or LGI1) and affect synaptic plasticity without causing major neuronal loss (3-5). Therefore, the neurologic alterations are potentially reversible by eliminating the antibodies or the cells that produce the antibodies (B cells, plasma cells) (6). Studies in the United Kingdom (7), The Netherlands (8), United States (9) or Spain (10) suggest that the encephalitis mediated by this category of antibodies directed against synaptic proteins or receptors are as frequent as herpes simplex encephalitis, and that anti-LGI1 encephalitis is the second most common antibody-mediated encephalitis after anti-NMDAR encephalitis (8). Because the treatment and follow-up of these patients usually require a wide spectrum of specialists in neurology, psychiatry, pediatrics, intensive medicine and rehabilitation, the impact of these diseases has been wide.

The current project will focus on anti-LGI1 encephalitis, which was discovered by our group in 2010. It mildly predominates in men (M:F, 6:4) and mostly older than 60 years (11, 12). The incidence is unclear; it has been estimated to be ~1 per million persons per year, but it is likely underestimated (8). All patients with anti-LGI1 encephalitis have IgG LGI1 antibodies (predominantly IgG4) in CSF and about 95% in serum (13). The disease has two distinct clinical phases: The acute phase in which the majority of patients develop severe short-term memory deficits (unable to remember events or experiences that occurred a few minutes earlier). This memory impairment can be preceded or accompanied by one or more of the following: hyponatremia (60% of patients), a highly distinctive type of seizures called facio-brachial dystonic seizures (~40% of patients), along with confusion, irritability and other types of focal seizures or less frequently, generalized seizures (11-15). In addition, many patients at this stage have symptoms of REM sleep behavior disorder (16). In this stage, the CSF may show pleocytosis or mild increase of proteins, the EEG is usually abnormal, and in ~60% of the patients the MRI shows typical increased FLAIR signal in medial temporal lobes (11). There is a clinical sub-phenotype (~13% of patients) in which the disease presents as a rapidly progressive cognitive decline without the indicated FLAIR MRI changes (15). About 70% of patients improve rapidly with corticosteroids and immunotherapy (eg, intravenous immunoglobulins and/or plasma exchange), but the improvement is often partial. After the acute phase, there is a chronic or residual phase which represents the interval from improvement of initial symptoms until the disease is considered no longer active and the remaining symptoms are thought to be irreversible. This chronic phase may take several months (it has been less well studied), and is characterized by the absence of CSF pleocytosis and inflammatory MRI changes (albeit this may show residual hippocampal atrophy), and very low or undetectable titers of serum antibodies. Most patients are unable to return to their job or previous activities due to residual (irreversible) memory or cognitive deficits accompanied by signs of moderate brain atrophy (14, 15). In addition, we and others have shown that about 27-35% of patients have relapsing symptoms after improving from the acute phase (14, 15). Although acute symptomatic seizures (facio-brachial dystonic and others) occur in ~90% of patients during the acute phase of the disease, less than 10% of patients develop chronic epilepsy often associated with hippocampal sclerosis (17). Therefore, the prevailing concept on this disease suggests a syndrome and clinical course in which the acute phase shows rapid, albeit partial, response to immunotherapy, and the symptoms of the chronic phase represent a burnout or irreversible process, in which the disease is no longer active, and the potential improvement of remaining symptoms is uncertain. As discussed later, we challenge this concept in the current proposal.

LGI1 is a neuronal secreted protein that forms a synaptic bridge, interacting at the presynaptic level with ADAM23 and the Kv voltage-gated potassium channels, and at the post-synaptic level with ADAM22 and the AMPA receptors (11). The protein has a leucine-rich domain and 7 repeats of about 50 amino acids (EPTP domains). We and others have shown that antibodies from patients with anti-LGI1 encephalitis react with epitopes contained in the leucine-rich domain and the EPTP domains (5, 18). Moreover, the indicated responsiveness to immunotherapy (compared with patients with classical paraneoplastic limbic encephalitis), and the demonstration that in cultured neurons patients' LGI1 antibodies disrupted the interaction of LGI1 with its synaptic partners (ADAM proteins) without causing neuronal death, suggested the pathogenicity of the antibodies (19). However, without an animal model it remained unclear whether the antibodies were able to cause clinical symptoms. We achieved this objective by adapting a mouse model (we previously developed for NMDAR antibodies) of passive transfer of patients' LGI1 antibodies (4). In this model, mice underwent a continuous (14 days) cerebroventricular infusion of patients' CSF enriched with LGI1 antibodies via

subcutaneously implanted osmotic minipumps connected with catheters to the lateral ventricles. Mice infused with patient's LGI1 IgG, but not control IgG, developed memory deficits which progressively recovered after the antibody infusion stopped. Furthermore, comprehensive confocal, patch-clamp and field potentials analyses in the CA1 region of the hippocampus showed that patients' IgG disrupt several LGI1-associated pathways, altering presynaptic and postsynaptic signaling, and causing neuronal hyperexcitability, decreased plasticity, and reversible memory deficits.

Clinical and immunological challenges pending to be solved

Although the advances described above are notable and were achieved in just a few years, there are still important gaps of knowledge related to the chronic phase of the disease and underlying immune-mechanisms that we are aiming to address in this proposal.

1- The concept that patients with autoimmune encephalitis respond rapidly to immunotherapy, and that most of the remaining symptoms a few months after disease onset are caused by irreversible brain alterations is inaccurate, as we recently reported for anti-NMDAR encephalitis (20). We postulate that the same occurs for anti-LGI1 encephalitis. In a recent prospective study in which 24 patients with anti-LGI1 encephalitis were invited to come to our center for additional clinical investigations during the chronic phase of the disease, we found that most (90%) had deficits in 1 or more of 6 cognitive domains at the first visit (V1), 65% had cognitive deficits at V2 (6 months), and 62% at V3 (1 year). In addition, at V1, about 90% of the patients had other symptoms (facio-brachial dystonic or focal seizures; REM sleep behavior disorder, insomnia) that were missed by patients, family members, or physicians. Some of these alterations were still present in 55% of patients at V2, and in 40% at V3. The main reason for the symptoms being unnoticed was that they often occurred at night or were identified with EEG or video-polysomnography.

Indeed, these studies showed that patients considered asymptomatic (regarding facio-brachial dystonic seizures, other seizures, or sleep disorder) had evidence of these types of symptoms with the appropriate tests. Because these clinical alterations were under-recognized, patients were no longer receiving immunotherapy or were undertreated with anti-seizure medications. These findings, which need to be confirmed and expanded with additional studies, are novel and important because they challenge the current prevailing view that facio-brachial dystonic seizures and other types of seizures, develop early in the course of the disease (often as first symptom) and resolve rapidly with immunotherapy (21). We also found that actimetry (or actigraphy assessed with a wrist-watch like device) may help to tract these nocturnal seizures at home, an observation that we propose to assess in the current proposal.

2- A question that remains to be answered for any type of autoimmune encephalitis is whether prompt cognitive rehabilitation can improve, reverse, or shorten the process of recovery. There is evidence that this type of rehabilitation improves the cognitive functions of patients with other disorders even in presence of irreversible neuronal damage (22), and there is preliminary data suggesting that it is also effective in patients with multiple sclerosis. Therefore, it is likely that cognitive rehabilitation will be effective in disorders such as the autoimmune encephalitides in which the alterations are mainly caused by neuronal dysfunction rather than by irreversible cell damage. For anti-LGI1 encephalitis (which typically occurs in patients older than 60 years and associates with protracted cognitive deficits) any improvement in cognitive functions would represent a major change in their quality of life, facilitating their return to previous baseline personal and professional activities.

3 - Modeling the disease in mice using active immunization with LGI1 peptides. For autoimmune encephalitis associated to antibodies against neuronal cell-surface proteins or receptors, the demonstration that patients' antibodies are pathogenic suggests that most of the symptoms are potentially reversible by removing the antibodies or antibody producing cells (memory B cells and plasma cells). In addition, knowing how the antibodies alter the protein targets (eg, antibody-mediated internalization of NMDAR for anti-NMDAR encephalitis,(6) or the indicated pre- and postsynaptic changes in LGI1-interacting proteins such as VGKC or AMPA receptors) may lead to novel treatment strategies such as allosteric modulation of receptor targets. We have demonstrated all these features in several animal models of passive cerebroventricular transfer of patients' antibodies, including NMDAR and LGI1 antibodies (8). However, despite the utility of these models, they do not fully reproduce the immunobiology of the disease and do not associate with inflammatory infiltrates. Moreover, passive infusion of antibodies to ventricles leads to a predominant diffusion of the antibodies to the hippocampus but not the entire brain, and have effects for only 12-15 days (until they are cleared from brain). To overcome these limitations we recently developed an active immunization model of anti-NMDAR encephalitis that we propose to adapt for anti-LGI1 encephalitis. Animals are immunized with selected peptides with highly immunogenic sequences along with the adjuvant Addavax (which primes B cell-mediated immune responses). Animals develop their own pathogenic antibodies, and brain inflammatory infiltrates allowing the assessment of how the autoimmune response leads to symptoms. It also, provides a chronic phase of the disease (71 days) during which different treatments can potentially be tested.

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3 Hypotheses of the study

The hypotheses of this proposal are:

1) Close follow-up and comprehensive studies (neurological, cognitive, epileptic activity, sleep dysfunction) will improve our understanding of the clinical phenotype of anti-LGI1 encephalitis, particularly the chronic phase. Determination of biomarkers of autoimmunity, inflammation, and neuronal degeneration in patients' serum will serve to follow-up disease activity.

2) Patients with anti-LGI1 encephalitis who receive prolonged cognitive training (eg, 6 months) will show better performance in memory and executive tests than patients who did not receive cognitive training (previously studied external control group).

3) A mouse model of active immunization with LGI1 peptides will reproduce better the disease than a previous model of passive transfer of antibodies that leads to antibody diffusion highly limited to hippocampal regions, absent inflammatory infiltrates, and changes that last for only 12-15 days. In contrast, an active immunization model will lead to inflammatory infiltrates and LGI1-specific B-cell responses involving the entire brain in association with: synthesis of LGI1 antibodies, synaptic alterations mediated by changes on LGI1 and interacting proteins, and behavioral alterations. We postulate that the alterations in all these paradigms will be long-lasting, reproducing not only the acute phase but also the chronic phase of the disease.

4 Objectives and aims of the study

The indicated hypotheses will be tested in **3 objectives**:

1) Determine the main clinical features (neurological, cognitive, EEG, video-polysomnography) of patients with anti-LGI1 encephalitis during the chronic phase of the disease, and assess serum biomarkers of autoimmunity/inflammation (LGI1 antibodies, RNA/NanoString analysis of targeted gene expression related to activation/function of B cells, T cells, microglia, and other interleukin/chemokine signaling) and neuronal degeneration (NfL levels).

2) Assess whether remote cognitive training during the first 6 months following initial hospital discharge modifies clinical outcome. Findings will be compared with a previously studied external control group of patients with anti-LGI1 encephalitis but who did not receive remote cognitive training.

3) Develop an animal model of active immunization using a repertoire of LGI1 peptides derived from the main immunogenic regions of LGI1. Determine the presence of LGI1 antibodies in serum/CSF and brain inflammatory infiltrates using immunohistochemistry. Examine how these immunological alterations alter the pre- and postsynaptic LGI1-interacting proteins (VGKC, AMPA receptor) and cause neurological symptoms; for this we will use confocal synaptic imaging, electrophysiology, and a standard panel of behavioral tests. In addition, we will assess the same serum biomarkers of autoimmunity/inflammation (RNA/NanoString analysis) as those used in patients, but with primers adapted to mouse.

4.1 Primary and secondary variables

1. Clinically characterize the post-acute stage of anti-LGI1 encephalitis, provide tools to remotely follow cognitive, behavioral and psychiatric deficits, and assess the impact of a cognitive rehabilitation program.

1.1. Cognitive assessment: Battery of 16 tests encompassing 8 cognitive domains: 1) intelligence quotient; 2) working memory; 3) learning and memory; 4) psychomotor speed; 5) executive functions; 6) selective and sustained attention; 7) language; 8) visuospatial perception. Total time ~2.5h.

1.2. Polysomnogram (PSG) will be adapted to patient's sleep habits (~23:00 to 07:30) using a digital polygraph (Deltamed). This includes EEG in 43 scalp channels + 11 channels for electrooculography, electrocardiography, electromyography, and audiovisual recording (sampling rate 256 Hz). Sleep stages will be scored manually (AASM criteria) using 30-s epochs, with modifications depending on sleep alterations. Data will be analyzed off-line in Python.

1.3. EEG (43 channels, 512 Hz) will be obtained the morning after the sleep study.

1.4. Brain MRI studies will be conducted on a 3 Tesla Prisma scanner using a 32-channel head coil. Scanning takes include 3D T1-weighted in sagittal plane; T2*axial EPI; axial diffusion weighted EPI; 3D sagittal FLAIR. MRI will not use contrast in the procedure.

1.5. Actimetry: at each presential visit, all patients will be provided with an actimeter (Actiwatch AW7, CamTech Ltd., UK), which is an accelerometer wrist-watch like device that measures movement, with the goal to determine whether at home it shows abnormalities that

correlate with some of the seizures and sleep alterations (eg, awakenings, sleep fragmentation) noted during the in person visit and video-polysomnography. In practical terms for patients, it will be akin to wearing a wristwatch (patients will leave the hospital with the device placed in the non-dominant wrist and in use, so that they will not need further instructions) for 1 week at home (day and night) after each presential visit (V1, V2 and V3). At the end of the week, a courier will collect it, and over the course of the following month, the recordings will be downloaded and analyzed with the MotionWare software (CamTech Ltd., UK). Actimetry is a test used in routine clinical care in the sleep disorders clinic and can be requested from the SAP request form. As with other tests, actimetry studies are identified with the patient's history number. Once the files are downloaded, in the case of research studies, they are identified with the patient's study code and stored in the F:/ folder. Only principal investigators can access the patient-code relationship.

1.6. Serum and CSF biomarkers of autoimmunity/inflammation and neuronal degeneration.
Left over samples of blood and CSF that were obtained and stored during the acute stage of the disease (by the time of symptom onset and local hospital admission) will be obtained from the core laboratory (CBD) for patients seen at our center or will be sent to our center from the referring doctors. The diagnosis of anti-LGI1 encephalitis requires demonstration of antibodies in CSF (serum can be negative); thus, initial CSF studies form part of standard of care in this disease. For the current study, the left-over routine care serum and CSF samples from the recruited patients will be used after their informed consent. For patients followed at our institution, we will take advantage of a regular follow-up visit to inform about the study and obtain their consent. For patients cared for at other hospitals, the referring doctors will explain the possibility of participating in the current study and, if the patients consent, we will contact them to provide further explanations and in such case obtain the informed consent. Serum and CSF collection procedures will be as performed in the routine clinical practice of these patients. These acute samples will be tested for the indicated (see below) biomarkers of autoimmunity, inflammation, and neuronal injury. We are a referral center for the diagnosis and study of this disease, and therefore we have ample experience in receiving and handling the samples.
During the post-acute stage (by the time that patients will come to Hospital Clínic de Barcelona), blood and CSF samples will be obtained for identical studies as those performed in the acute stage serum and CSF samples. Two lumbar punctures will be performed, after informed consented, one at the first presential visit (V1) and the other at the last presential visit (V3).. Based on a recently published pilot study and extensive experience with the disease, all patients at V1 have cognitive, sleep and epileptic alterations and most still have residual deficits at V3; thus, the CSF studies are important for investigations on biomarkers of disease severity, outcome and prognosis, and for better optimization of therapeutic options. They include:

- LGI1 Antibodies, determined with brain tissue immunohistochemistry and cell-based assays.
- HLA genotyping, performed by standard techniques based on DNA-PCR and polymorphism identification by reverse hybridization with specific probes and fluorescence labelling of hybridized fragments (PCR-SSOP) (Immucor GTI Diagnostics Inc. Waukesha USA) in combination with genomic DNA sequencing by Sanger methodology (PCRSBT).
- RNA/NanoString analysis of targeted gene expression related to activation/function of B cells, T cells, microglia, and other interleukin/ chemokine signaling. Whole blood/CSF will be collected using PAXgene® Blood RNA tubes (Qiagen) shipped to the centers. Total RNA will be extracted using PAXgene® Blood RNA Kit (Qiagen). RNA samples are quantified using Qubit 2.0 Fluorometer (Life Technologies) and RNA integrity is determined with Agilent 2100 Bioanalyzer

(Agilent Technologies). Expression levels of 44 genes related to immunological pathways and cytokines (Annex,Table) will be measured with the nCounter® Digital Analyzer (NanoString), as reported (*Armangue et al., Mol Genet Metab 2017;122:134-9*). Twenty healthy participants will serve as controls (single evaluation).
- NfL levels, determined in serum and CSF using the SiMoA Quanterix technique, as reported (*Guasp et al., Neurology 2022;98:e1489-98*). Age- and sex-matched healthy participants from previous studies will serve as controls. These studies will provide a signature of molecular biomarkers in the acute and chronic stages. We expect they will correlate with the clinical features assessed in Aim1 and will assist in treatment decisions.

1.7 Autonomic nervous system (ANS) evaluation

Cardiovagal evaluation: Continuous electrocardiogram recording using surface electrodes will monitor changes in heart rate during deep breathing at 6 cycles per minute, Valsalva maneuvre and bipedestation.

Sympathetic adrenergic evaluation: Continuous blood pressure (CNS systems) and manual pressure recording will monitor changes during Valsalva maneuvre, isometric activity and standing up (or passive inclination if the subject is unable to remain standing up without support).

Sympathetic cholinergic evaluation: Using surface electrodes applied to the hand and foot of the subject we will record the change of skin conductance to low-intensity electrical stimuli on the median or posterior tibial nerve, deep inspiration and startle (sympathetic skin response). A specific questionnaire for the assessment of dysautonoic symptoms (Composite Autonomic Symptoms Scale COMPASS 31) will be administered.

1.8 Electromyography (EMG).

A little needle recording electrode will be inserted into different muscles from cranial (orbicularis oris), cervical (extensor indicis propius) and lumbar (tibialis anterior) segments. Electrical signal from individual muscle fibers and motor units both at rest and during voluntary contraction will be recorded.

1.9 Brainstem reflex

Trigeminal blink reflex, mediated by trigemino-facial ponto-medullary-circuits will be assessed. Surface recording electrodes will be attached over the *orbicularis oculi* in both sides with active electrode over the middle part of the lower eyelid and the reference at the lateral cantus of the eye. Stimulating electrodes will be placed over the supraorbital nerve. Ipsilateral (R1, R2) and contralateral responses (R2c) latencies measured in ms will be analyzed.

2) Development an animal model of anti-LGI1 encephalitis using active immunization

2.1. Behavioral studies. From baseline (pre-immunization) until day 71 (euthanized) animals will be regularly tested with an extensive panel of memory/behavioral paradigms, as reported in our model of passive transfer of antibodies (*Petit-Pedrol et al., Brain 2018;141:3144-59*).

2.2. Effects on LGI1. The antibody effects will be determined in subsets of mice euthanized at day 42 (acute phase) and the rest of mice on day 71 (chronic phase). Sections of cortex/hippocampus will be examined using (1) confocal microscopy and IMARIS software to quantify the total surface and synaptic clusters of LGI1 and their synaptic protein partners (Kv1.1 and AMPA receptor), and (2) electrophysiology to assess hippocampal long-term (LTP) and short-term potentiation (STP). These techniques were described in detail in our passive immunization models of NMDAR and LGI1 antibodies (*Planagumà et al., Brain 2015:138:94-109; Petit-Pedrol et al., Brain 2018;141:3144-59*).

2.3. Immune cells and immune-inflammatory pathways. From tissue samples obtained on days 42 and 71, immune/ inflammatory cells (B, T, activated microglia, etc.) will be examined by immunohistochemistry with antibodies specific for the indicated cell types, as reported (Planagumà et al., Brain 2015:138:94-109). RNA will be extracted from brain for RNA/NanoString analysis targeting the same genes as described in patients (see Annex Table), with mice-RNA primers. Findings from the acute stage (day 42) will be compared with the post-acute stage (day 71).

5 Design of the study

Human studies

Clinically characterization of the post-acute stage of anti-LGI1 encephalitis: Prospective single center, single arm, national study of 3 years duration.
Our already established multidisciplinary team has extensive experience in developing similar studies on patients with autoimmune encephalitis, and the results have been so far satisfactory from both a scientific perspective and for patients. In specific, the study will be conducted following the protocol that we developed for previous prospective longitudinal observational studies performed between 2017 and 2022 with patients with anti-NMDAR encephalitis during the post-acute stage (**Study title: "Immune-mediated diseases of the synapse: symptoms, brain networks, and the link to human memory"; PI: Josep Dalmau; Code: HCB/2016/0596)**, and with patients with anti-LGI1 encephalitis during the post-acute stage **(Study title: "A Translational Model of Antibody-mediated Synaptic Disease: Symptoms, Neuronal Circuits, and the Mechanisms of Memory Loss and Recovery"; PI: Josep Dalmau; Code: HCB/2018/0561)**, that will serve as the control group. No new visits will be necessary in this control group.
The cognitive rehabilitation program will be implemented following the same protocol and tools that we are currently using in a very similar study but with patients with anti-NMDAR encephalitis (**Study title: "Antibody-mediated NMDA receptor encephalitis: symptoms, biomarkers, and mechanisms of the prolonged recovery stage"; PI: Josep Dalmau; Code: HCB/2022/1070)**. All of them had been accordingly approved by the CEIm of HCB.

Development an animal model of anti-LGI1 encephalitis using active immunization

Experimental design. Mice C57BL/6J, 8-10 weeks old will be used. All mice will be immunized twice (days 0 and 28) with a mixed combination of selected LGI1 peptides (see Annex text, and Annex Fig E). As adjuvant, we will use Addavax+Pertussis toxin that primes B-cell and antibody responses with better tolerability than Freund's adjuvant. In an experimental model of anti-NMDAR encephalitis, this approach resulted in very high titers of NMDAR antibodies with

reactivity similar to that of patients' antibodies (see first section, "Previous Activitaties"). The corresponding control group will receive only Addavax+Pertussis toxin without peptide. The total number of mice including those for the selection of peptides, controls, sets of animals sacrificed on days 42 and 71, and subsets needed for acute brain slices for electrophysiology and fresh tissue for NanoString/RNA studies is 104.

6. Selection of participants

1.1. Patient recruitment and number of patients. 20 LGI1 patients will be recruited: families and patients will be approached during the acute phase of the disease and invited to participate in the study after hospital discharge.

Identification of patients will be done in (1) our hospital, (2) through a network of Spanish hospitals (already in place), and (3) the Neuroimmunology lab at HCB/IDIBAPS (referral center for autoimmune encephalitis). Informed consent and general information through a structured questionnaire will be obtained from participants/legal guardians. Regarding the number of patients, we have selected 20 patients based on our published experience with another autoimmune disease (NMDAR encephalitis) and a previous pilot study with anti-LGI1 encephalitis (used as external control group who did not have remote cognitive training). In both studies, the number of ~20 patients was informative.

Exclusion criteria include: patients unable to travel to our center, and previous history of psychiatric or neurologic diseases (eg, schizophrenia, dementia, stroke) that may interfere with the proposed studies. All patients will have a preliminary assessment of their interest in participation and any of the indicated incompatibilities

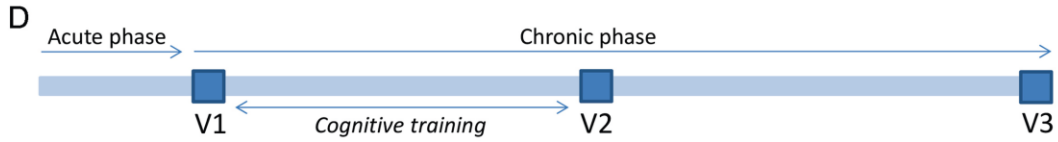
7. Treatment and calendar of the study

In-person visits include 3 visits during the post-acute stage:
V1 (after hospital discharge, ~1-3 weeks after symptom onset),
V2 (6 months),
and V3 (1 year) (Fig D).

Each visit consists of ~1 day and 1 night at Hospital Clínic (distributed in 1/2 day, 1 night, and 1/2 day) during which they undergo clinical assessments and tests according to a pilot study: neurologic and cognitive assessment, EEG, ANS, EMG, brainstem reflex study and video-polysomnography. At V2 we will obtain an MRI (to compare with that of the acute phase). We will also obtain and the blood and serum. During the post-acute stage (by the time that patients will come to Hospital Clínic de Barcelona), blood and CSF samples will be obtained for identical studies as those performed in the acute stage serum and CSF samples. Two lumbar punctures (LP) will be performed, after informed consented, one at the first presential visit (V1) and the other at the last presential visit (V3). After the first visit, patients/families will be instructed to keep remote cognitive training for 6 months. The frequency of cognitive training is suggested by data from our pilot study in which 90% of patients had cognitive abnormalities at V1 and the improvement (albeit partially; without cognitive training) mainly occurred during the first 6 months. These patients will serve as external controls.

Summary:
V1: neurologic and cognitive assessment, EEG, ANS, EMG, brainstem reflex study and video-polysomnography. Blood and serum examination. Lumbar puncture

V2: neurologic and cognitive assessment, EEG, ANS, EMG, brainstem reflex study and video-polysomnography. MRI. Blood and serum examination
V3: neurologic and cognitive assessment, EEG, ANS, EMG, brainstem reflex study and video-polysomnography. Blood and serum examination. Lumbar puncture



Presential visits

- Presential standard neuropsychiatric examinations and cognitive assessments will be conducted as per routine clinical practice and with a reported panel of neurological functional, cognitive and psychiatric tests. Total time ~4h spread over 3 visits (the exact timetable can be adapted to patients' preferences and the length of the interviews will vary according to patients' clinical status; the times are indicative and are overestimated to be sure). These clinical evaluations will take place in the same room where the patients will sleep, and patients will have enough pauses in between to rest, eat/drink and go for a walk outside the hospital with their relatives.

- Psychophysical testing of working memory: The duration of this task depends on the performance of the patients; maximum ~1:30h. It will be done in the same room during V1 (1st visit in our center), V2 (6 months follow-up) and V3 (1 year follow-up).

- MRI studies. It is a passive examination, patients will not need substantial mental or physical efforts. Total time ~45min during V2 (6 months follow-up).

- PSG: It is a passive examination, patients will not need substantial mental or physical efforts. Patients will sleep in the same room where the other clinical examinations and psychophysical testing will be carried out, so they will get used to and adjust to the environment during the day. Total time: night sleep during V1 (1st visit in our center), V2 (6 months follow-up) and V3 (1 year follow-up).

- ANS: It is an active examination. Patients will perform simple and non invasive exercises regarding deep breathing, blowing and standing up.

- EMG: It is a passive examination, patients will not need substantial mental or physical efforts.

- Brainstem reflex: It is a passive examination, patients will not need substantial mental or physical efforts.

- EEG: It is a passive examination, patients will not need substantial mental or physical efforts. Total time ~45min during V1 (1st visit in our center), V2 (6 months follow-up) and V3 (1 year follow-up).

- Actimetry: It is a passive examination, patients will not need substantial mental or

physical efforts. Total time: one week after V1 (1st visit in our center), V2 (6 months follow-up) and V3 (1 year follow-up).

- Lumbar Puncture will be performed, after informed consented, one at the first presential visit (V1) and the other at the last presential visit (V3).

- Brain recordings - It is a passive examination, patients will not need substantial mental or physical efforts. Total time: night sleep.

- Patients' blood and serum will be examined at the time of diagnosis (sent from local hospitals to our center via courier) and at the post-acute stage: V1 (1st visit in our center), V2 (6 months follow-up) and V3 (1 year follow-up). It will be performed in the same room; total time ~30min

Remote cognitive rehabilitation program will be performed through an online validated platform (Guttmann Neuropersonal Trainer [GNPT]: https://gnpt.es/) run by our team. This is a Sanitary Product with CE certification (Producto Sanitario RPS/430/2014; International Patent [PCT/ES2008/00677]) and here will be used within its approved indications. The rehabilitation program will increase in difficulty and decrease in frequency during the six months of follow-up (V1-V3).

Patients/families will be instructed to perform remote cognitive rehabilitation during the presential visits. Patients will be able to use their personal computer and access via an available link and a user code the GNPT platform. In case that they do not have easy access to adequate equipment to access the platform, we will provide the iPad tablet with all the necessary software installed and ready to use to support their training. The indicated frequency of cognitive training is based on results of a recently published pilot study in which patients were followed with only presential visits (no remote visits or cognitive training was provided). The study showed that a slow progressive cognitive improvement occurred in most patients during the 1 year follow-up. However, the improvement was more marked during the first 6 months compared with the last 6 months. This finding suggested that intensive cognitive training (particularly during the first 6 months) could accelerate recovery and improve outcome. Accordingly, for the current study patients will undergo cognitive rehabilitation twice per week for the first 3 months and once per week for the last 3 months. Similar programs are currently used by our team for patients with multiple sclerosis (along with collaborations with Institute Guttmann, Barcelona). Remote cognitive sessions will take ~45-60min.

8. Statistics

Human studies: Cross-sectional comparisons among the 2 groups of patients will be done with Analysis of Covariance (ANCOVA) and Chi-Squared tests, as appropriate. Post-hoc analyses with Bonferroni correction for post-hoc multiple comparisons will be applied to all analyses. Analysis of inter- and intragroup differences in the longitudinal follow-up will be done using multilevel linear mixed-effect (LME). Lme4 R package (version 1.1-27.1) will be used to fit LME models. Comparisons will be done with emmeans library (version 1.7.3) and Tukey method for post-hoc correction for multiple testing. The global tendency of recovery of cognitive deficits in patients receiving or not cognitive training will be assessed with the Cochran-Armitage test for trend. All analyses will be addressed considering a two-tailed type I error of 5% with statistical

significance set at p-value <0.05, using SPSS (version 25; IBM Corp, Armonk, NY) and R package (version 4.1.2; Vienna), as previously reported (*Guasp et al., Lancet Neurol 2022;21:899-910*).

Animal studies: Animal behavior with multiple determinations in time (e.g., recognition of new object) will be analyzed using repeated measures two-way analysis of variance (ANOVA). Behavioral studies with a single time determination (e.g., depressive behavior) will be analyzed with one-way ANOVA or Mann-Whitney-U for skewed distributions (e.g., tail suspension). The intensity of IgG deposits in brain and electrophysiological studies (long-term potentiation) will be analyzed with two-way ANOVA. The reactivity intensity of the antibodies of patients in HEK cells (CBA), neuron cultures, or brain tissue, or quantification of synaptic markers (e.g., GluN1, PSD95) or activated microglia, in a single time determination, will be analyzed by one-way ANOVA. Post-hoc analyses for all experiments will use Bonferroni correction for multiple testing. A p value of <0.05 will be considered significant and the α-error will be set to 0.05. Analyses will be done with GraphPad Prism v7 (*Planagumà et al., Ann Neurol 2016;80:388*).

9. Risks and benefits for participants in the study

It is possible that patients will not benefit from participating in this study. However, based on the results of previous similar studies ("Immune-mediated diseases of the synapse: symptoms, brain networks, and the link to human memory", IP: Josep Dalmau, Code: HCB/2016/0596; "A Translational Model of Antibody-mediated Synaptic Disease: Symptoms, Neuronal Circuits, and the Mechanisms of Memory Loss and Recovery", IP: Josep Dalmau, Code: HCB/2018/0561) we expect that patients will directly and immediately benefit from their participation in the carefully monitored cognitive training program and the close clinical follow up in an experienced unit.

Our previous studies with this same load of tests in presential visits were successful and patients with anti-NMDAR and anti-LGI encephalitides returned for the most part in successive retest sessions after 3, 6, 12 and even 24 months (Guasp et al. Lancet Neurology 2022; Ariño et al. Neurology 2020; Stein et al. Nat Commun 2020). This demonstrates that the load of tasks and frequency of visits was not unbearable and patients were happy to maintain participation in the project for an extended period of time and repeat sessions with identical task loads. We specifically gauged participant satisfaction in a gathering of participating families at Hospital Clínic in 2018, where we presented the objectives and initial results of the study and allowed participants to ask questions and express their concerns. Participants were very satisfied with the study and appreciated the opportunity to be monitored in the reference center for this disease in Europe, and to get in touch with other families afflicted by the same rare disease. We plan to repeat this experience in the framework of the present project in order to detect possible dissatisfactions with our experimental protocols and adapt them for the comfort of the participants.

Nonetheless, the detailed risks for each of the procedures in the current study are as follows:

a) Risks of presential Neurologic, Psychiatric and Cognitive assessments: These evaluations are innocuous and do not represent any substantial risk for participants. The main risk of these evaluations is fatigue, and they may be demanding in terms of execution time, but patients will be able to take breaks during the testing to alleviate it. Moreover, they will be guided by a member of our team to ensure that it does not cause any major discomfort to the patient.

b) Risks of Magnetic Resonance Imaging (MRI): The known risks associated with MRI studies are minimal. The procedure uses radio waves and a magnetic field to take pictures. Some individuals may feel claustrophobic (uncomfortable in small areas) during the MRI scan or may be disturbed by the sounds of the machine (which are loud and repetitive). Patients can be given music to listen to during the scan or can hear the voice of the technician. These have been shown to help patients relax during the scan. If the subject experiences these feelings and is uncomfortable, they can always discontinue the scan

c) Risks of presential EEG studies: EEG is considered a safe procedure and causes no discomfort. During the recording, subjects can relax. Some people may develop a mild rash where the electrodes were attached that resolves without treatment.

d) Risks of presential ANS, EMG and brainstem reflex: These neurophysiological tests are considered safe procedures which are performed daily in our centre. Discomfort related to electrical stimulation and needle insertion is minimal.

e) Risks of presential Sleep studies: The same for EEG studies, as well as possible discomfort before falling asleep and reduced night rest. However, the cabling system is optimized to facilitate the free movement of patients at night, and the room is soundproofed.

f) Risks of Psychophysical testing and Brain recordings: These evaluations are innocuous and do not represent any substantial risk for participants. The main risk of these evaluations is fatigue, reduced night rest, and they may be demanding in terms of execution time. However, they will only be performed in the course of two weeks separated by 6 months, for each participant. The computerized task will have a game component to make it engaging, as well as the device is specifically designed to be comfortable during sleep for nocturnal recordings. In the first day of the week of recordings, a member of our team will make a video-conference with the participant (including family members if they are minors) to assist with the setup of the device for testing and recording.

g) Risks of Blood Tests: Risks associated with blood drawing include infection, bleeding, bruising, fainting and discomfort.

h) Risks of Lumbar Puncture: Although lumbar puncture (spinal tap) is generally recognized as safe, it does carry some risks. These include post-lumbar puncture headache that can last from a few hours to a week or more. Lying flat and drinking fluids helps to relieve this headache. Patient may experience temporary back discomfort or pain, and mild bleeding that may occur near the puncture site. These side effects resolve without treatment. It is possible that patients will not benefit from the lumbar puncture, but for those patients with unfavourable clinical evolution or relapse, NMDAR antibody testing in CSF will help guide therapeutic decisions (antibody titters in CSF, but not in serum, have clinical correlation).

9. Ethics and legal aspects

The study will be carried out in compliance with the Helsinki Declaration (current version, Fortaleza, Brazil, October 2013) and in accordance with the protocol and with the relevant legal requirements, which in this case is Biomedical Research Law 14/2007 of July 3, and Regulation 2017/745 on Medical Products. Informed consent will be obtained for all patients before their inclusion in the study, and their samples stored in the IDIBAPS-Hospital Clínic Neuroimmunology Laboratory until they are used for the objectives of this study.

10. Data management

Clinical data from patients will be coded. Each patient will be assigned a code. In another

separate database, the IP will store the relationship between each code and the patient's medical record number.

Coded information from patients will be stored in online Redcap database from HCB (redcap.clinic.cat).

Computers and wearables will be stored at Cellex 3A, Neuroimmunology laboratory.

11. Treatment of data and archiving of registers. Confidentiality of the data

The treatment, communication and transfer of personal data of all participants will comply with EU Regulation 2016/679 of the European Parliament and of the Council of April 27, 2016 regarding the protection of natural persons in terms of to the processing of personal data and the free circulation of data, being mandatory as of May 25, 2018. The legal basis that justifies the processing of your data is the consent given in this act, in accordance with the provisions of the Article 9 of EU Regulation 2016/679.

The data collected for these studies will be collected identified only by a code, so no information will be included that would allow the participants to be identified. Only the study doctor and his collaborators with the right of access to the source data (clinical history), may relate the data collected in the study with the patient's medical history. In the future, the data collected and properly coded may be used also to address other questions relevant for the understanding of anti-LGI1 encephalitis, and we will request specific consent for this potential prospective use of the data.

The identity of the participants will not be available to any other person except for a medical emergency or legal requirement.

The health authorities, the Research Ethics Committee and personnel authorized by the study promoter may have access to the identified personal information, when necessary to verify data and study procedures, but always maintaining confidentiality in accordance with current legislation. .

Only coded data will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). In the event that this transfer occurs, it would be for the same purpose of the study described and guaranteeing confidentiality.

If a transfer of encrypted data takes place outside the EU, either in entities related to the hospital center where the patient participates, to service providers or researchers who collaborate with us, the data of the participants will be protected by safeguards such as contracts or other mechanisms established by the data protection authorities.

As promoters of the project, we undertake to process the data in accordance with EU Regulation 2016/679 and, therefore, to keep a record of the treatment activities that we carry out and to carry out a risk assessment of the treatments we carry out, to know what measures we will have to apply and how to do it.

In addition to the rights that the previous legislation already contemplated (access, modification, opposition and cancellation of data, deletion in the new Regulation) now participants can also limit the processing of data collected for the project that are incorrect, request a copy or that moved to a third party (portability). To exercise these rights, they should contact the main researcher of the study or the Data Protection Delegate of the Hospital Clínic de Barcelona through protecciodades@clinic.cat. They also have the right to

contact the Data Protection Agency if they are not satisfied.

The data cannot be deleted even if a patient leaves the study, to ensure the validity of the research and to comply with legal duties and drug authorization requirements.

The Researcher and the Sponsor are obliged to keep the data collected for the study at least until 5 years after its completion. Subsequently, personal information will only be kept by the city center or their health and by the promoter for other purposes of scientific research if the patient has given their consent to do so, and if this is permitted by law and applicable ethical requirements.

12. Management of biological samples

The serum of the patients will be stored in the Neuroimmunology Tissue Collection (registered in the ISCII with number C0000051 and in the Biobank of IDIBAPS with the number R091217-012). All patients consent to their inclusion in this collection. The samples are coded so that the samples can be related to the clinical information but not to the donor patient.

13. Funding

FIS ISCIII- PI23/00858 granted.

14 Publication policy

We commit to making public the results of the study, whether they are positive or negative.

ADDENDUM:

The Spanish anti-LGI1 encephalitis study group:

Aguilar, Lorena	Hospital de la Santa Creu i Sant Pau, Barcelona, Spain	lmartina@santpau.cat
Álvarez, Gary	Hospital Universitaride Girona Doctor Josep Trueta, Girona, Spain	garyalva-rez.girona.ics@gencat.cat
Alvarez, Marta	Hospital de Mataró, Mataró, Spain	malvarezla@csdm.cat
Anguita, Alicia	Hospital Sant Joan de Déu (Althaia), Manresa, Spain	aanguita@althaia.cat
Arroyo, Francisco	Hospital Nuestra Señora del Rosario, Madrid, Spain	fgilo21@gmail.com
Baron, Manuel	Hospital Universitario Fundación Alcorcón, Madrid, Spain	Mbaron@fhalcorcon.es
Bataller, Luis	Hospital Universitari i Politècnic La Fe, Valencia, Spain	bataller_lui@gva.es
Bonet, Macarena	Hospital Universitari i Politècnic La Fe, Valencia, Spain	bonet_mac@gva.es;
Caballero, Eva	Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain	ecaballe@clinic.cat
Clares, Rocío	Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain	rociohclares@hotmail.com
Coll, Cristina	Hospital Universitaride Girona Doctor Josep Trueta, Girona, Spain	ccoll.girona.ics@gencat.cat
Cruz, Hector	Consorci Hospitalari de Vic, Vic, Spain	hektorkruz@gmail.com;
Erro, Elena	Complejo Hospitalario de Navarra, Pamplona, Spain	elena.erro.aguirre@navarra.es
Estela, Jordi	Parc Taulí Hospital Universitari, Sabadell, Spain	jestela@tauli.cat
Font, Maria	Consorci Hospitalari de Vic, Vic, Spain	mafont@chv.cat
Franquet, Elisabet	Hospital Residència Sant Camil, Barcelona, Spain	efranquet@csg.cat
Gil, Fran	Hospital Universitari Sagrat Cor, Barcelona, Spain	fj.gil@quironsalud.es
Larsen, Alvaro	Complejo Hospitalario Universitario de Albacete, Albacete, Spain	aa.sanchezlarsen@gmail.com
Hernández Lorido, Raquel	Hospital Marina Baixa La Vila Joiosa, Villajoyosa, Spain	hernandez_raq@gva.es
Madera, Jorge	Hospital Universitario Marqués de Valdecilla, Santander, Spain	jorge.madera@scsalud.es
Mateo, Jose	Hospital Universitario Marqués de Valdecilla, Santander, Spain	joseignacio.mateo@scsalud.es
Matos, Nuria	Hospital Sant Joan de Déu (Althaia), Manresa, Spain	nmatos@althaia.cat
Mendez, Carla	Complejo Hospitalario Universitario de Huelva, Huelva, Spain	carlamendezbarrio@gmail.com
Monterda, Angela	Hospital Universitari Joan XXIII, Tarragona, Spain	amonterde.hj23.ics@gencat.cat
Moreira, Antia	Hospital Universitari d'Igualada, Igualada, Spain	amoreira@psmar.cat
Morejon, Fernando	Hospital IMED Levante, Benidorm, Spain	fjmore-jon@imedhospitales.com
Muñoz, Alvaro	Hospital Royo Villanova, Zaragoza, Spain	alvaro.gimenezm@gmail.com
Ortega, Fernando	Hospital Universitario Marqués de Valdecilla, Santander, Spain	fernando.ortega@scsalud.es
Querol, Luis	Hospital de la Santa Creu i Sant Pau, Barcelona, Spain	lquerol@santpau.cat
Riancho, Javier	Hospital Universitario Marqués de Valdecilla, Santander, Spain	javier.riancho86@gmail.com
Solà, Nuria	Hospital Universitari Sant Joan de Reus, Reus, Spain	nuria.sola@gmail.com
Toledano, Rafael	Hospital Universitario Ramon y Cajal, Madrid, Spain	rafatoledano@hotmail.com
Vega, Álvaro	Hospital Universitari Son Llatzer, Mallorca, Spain	avega@hsll.es
Vidal, Anna	Hospital Sant Joan de Déu (Althaia), Manresa, Spain	amas@althaia.cat
Viñas, Jaume	Hospital Universitari Joan XXIII, Tarragona, Spain	jvinas.hj23.ics@gencat.cat