

Supplemental information

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 isothiocyanate (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 ^{125}I - 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 ^{125}I - 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.

Reference List

- (1) Ances BM, Vitaliani R, Taylor RA, et al. Treatment-responsive limbic encephalitis identified by neuropil antibodies: MRI and PET correlates. *Brain* 2005 Aug;128:1764-1777.
- (2) Lancaster E, Huijbers MG, Bar V, et al. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann Neurol* 2011 Feb;69:303-311.

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	Green	Green	Orange	Orange	Orange	Orange	Green
24	M	76	No	3	7	Green	Orange	Green	Orange	Green	Orange	Orange
25	M	66	No	3	7	Green	Orange	Orange	Green	Orange	Green	Orange
26	M	40	No	3	7	Green	Orange	Orange	Orange	Green	Orange	Green
27	M	74	No	2	3	Green	Green	Orange	Grey	Grey	Grey	Grey
28	M	25	Yes	2	3	Grey	Grey	Green	Orange	Grey	Green	Grey
29	M	72	Yes	2	5	Green	Orange	Orange	Grey	Grey	Orange	Green
30	M	60	Yes	2	7	Green	Orange	Orange	Orange	Orange	Green	Orange
31	M	72	N.A.	1	7	Green	Orange	Orange	Orange	Orange	Orange	Orange
32	M	68	Yes	2	7	Green	Orange	Orange	Orange	Green	Orange	Orange
33	M	75	Yes	1	6	Green	Orange	Orange	Orange	Grey	Orange	Orange
34	M	74	No	2	7	Green	Orange	Orange	Orange	Orange	Green	Orange
35	M	62	No	1	6	Green	Orange	Orange	Orange	Grey	Orange	Orange
36	F	58	Yes	1	4	Green	Orange	Orange	Grey	Orange	Grey	Grey
37	M	67	N.A.	1	4	Orange	Grey	Grey	Orange	Grey	Green	Orange
38	M	58	No	1	3	Green	Orange	Orange	Grey	Grey	Grey	Grey

Subacute onset = progression to maximum disease severity in three months

Green = symptom present. Orange= symptom absent. Grey striped = 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	Green	Green	Orange	Green	Green	Orange	Orange	Creutzfeldt Jakob disease
2	F	28	No	3	7	Orange	Orange	Green	Orange	Green	Green	Orange	Cramp fasciculation syndrome
3	M	56	Yes	2	2	Green	Grey striped	Grey striped	Grey striped	Green	Grey striped	Grey striped	Autoimmune encephalopathy
4	M	63	No	2	6	Green	Grey striped	Orange	Orange	Orange	Orange	Green	Encephalopathy with hypokinetic rigidity
5	M	78	No	2	7	Green	Green	Orange	Orange	Orange	Orange	Orange	Creutzfeldt Jakob disease
6	F	62	Yes	1	2	Green	Grey striped	Orange	Grey striped	Grey striped	Grey striped	Grey striped	Psychiatric symptoms
7	F	37	Yes	1	2	Green	Grey striped	Grey striped	Orange	Grey striped	Grey striped	Grey striped	Neuro-SLE
8	F	83	Yes	1	2	Green	Grey striped	Grey striped	Grey striped	Grey striped	Orange	Grey striped	Episodic cognitive disturbance
9	M	62	No	1	7	Green	Orange	Orange	Orange	Orange	Orange	Orange	Limbic encephalitis
10	M	78	No	1	7	Green	Orange	Orange	Orange	Orange	Orange	Orange	Limbic encephalitis

Subacute onset = progression to maximum disease severity in three months

Green = symptom present. Orange= symptom absent. Grey striped = symptom not documented.

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

