**Appendix e-1**

*Indirect immunofluorescence assay (IFA)*

Patient serums, absorbed with bovine liver powder to remove non organ-specific IgG (1:240), and CSFs (unabsorbed, 1:2), were tested on cryosections of composite adult mouse tissues: cerebellum, midbrain, cerebral cortex, hippocampus, thalamus, hypothalamus, kidney and gut as described previously.1, 2

*Protein purification and Sequencing*

*Antigen Preparation*

Cytosolic and membrane preparations were used. For the cytosolic antigen preparation, adult mouse cerebellum and cerebrum were homogenized with buffer, 2ml/g (10mM Hepes, 1mM MgCl2, 1mM ethylenediaminetetraacetic acid, and complete protease inhibitor cocktail [Roche, Indianapolis, USA]). Homogenate was clarified by centrifugation (100000 x g, 30 minutes) and supernatant was stored at -80°C. For the membrane preparation, pellet from the cytosolic preparation was re-suspended in NP-40 buffer (1% NP-40, 0.1%SDS, 0.15M NaCl, 0.01M NaPO4 pH 7.2, 2mM EDTA, and complete protease inhibitor cocktail [Roche, Indianapolis, USA]) for 2 hours then centrifuged at 100000 X g for 30 minutes. Supernatant was stored at -80°C.

*Western blotting, Immunoprecipitation, & Mass Spectrometry*

For western blot, mouse cytosolic or membrane cerebellar extract was separated on 4-15% or 10% polyacrylamide gels, and the membrane was probed with sera from healthy subjects and patients (1:200 dilution), and commercial cotactin-1 IgG (1:500).

A candidate antigen was identified by protein G magnetic bead capture from mouse cerebral lysate using sera from two patients with unique synaptic staining pattern. Serum IgGs from 2 patients and 1 healthy control were complexed to protein G magnetic beads (Dynabeads; Invitrogen, Thermo Fisher Scientific [Waltham, MA]) and exposed to mouse cerebellar protein extract (20 minutes, at room temperature). After washing, beads were boiled for 5 minutes in 2 × Laemmli sample buffer. The eluted proteins were electrophoresed in 5% polyacrylamide gel, and located by Coomassie G-250 staining (Bio-Rad, Hercules, CA) and by western blot. Bands corresponding to immunoreactivity were excised and analyzed by high-pressure liquid chromatography electrospray tandem mass spectrometry (Mayo Clinic Medical Genome Facility—Proteomics Core). Mass spectrometry analysis of the electrophoretically separated proteins predicted the 130 kDa protein to be contactin 1.

*Antibody purification*

Mouse cerebellar proteins were separated electrophoretically in 10% polyacrylamide gel (SDS-PAGE), and were transferred electrophoretically to a nitrocellulose membrane. Patient IgG bound to the membrane at the target molecular weight were acid-eluted and used for IFA, as previously described.1

*Recombinant protein western blot*

Glutathione S-transferase (GST)-tagged recombinant contactin 1 full-length protein (R&D systems, catalog# 9665-CN, USA) and caspr 1 overexpression lysate (Origene, catalog# LY401200 USA) was separated on 4-15% polyacrylamide gels, transblotted to nitrocellulose membranes and probed with serum IgGs from healthy subjects or patients (1:500 dilutions).

*Cell-binding assay (CBA)*

Contactin-1 specificity was confirmed by indirect immunofluorescence on HEK293 cells transfected with full-length contactin 1 complementary DNA. Control cells in two different chambers were transfected with neurofascin 155 complementary DNA and empty vector. One additional chamber was co-transfected with both contactin 1 and caspr 1 complementary DNA. Cells were grown on glass coverslips, fixed with ice-cold formalin, and prepared as millimeter-sized biochip fragments on microscope slides as a mosaic of the following co-expressions: contactin-1 and neurofascin-155; contactin-1 and caspr1; and, caspr1 expressing control cells (Euroimmun AG, Lubeck, Germany). Patient or control serum (1:200 dilution), CSF (1:10) or commercial commercial-5-IgG (1:500) were added to the cells for 45 to 60 minutes at room temperature. Secondary antibody (FITC–conjugated goat anti-human, anti-rabbit or anti-mouse IgG, 1:400) was applied for 30 minutes.Contactin-1 IgG4 subclass was identified using subclass‐specific secondary antibodies (mouse anti‐human IgG4‐Fc‐specific FITC‐conjugated [Southern Biotech]).

*Assays for defined neural autoantibodies*

Serum and CSF were tested by standardized indirect immunofluorescence assay on a composite substrate of mouse cerebellum, midbrain, basal ganglia, thalamus, cerebral cortex, hippocampus, stomach and kidney to detect IgG autoantibodies binding selectively to neuronal and glial nuclei (antineuronal nuclear antibodies [ANNA], type 1 [anti Hu], type 2 [anti Ri] and type 3; antiglial/neuronal nuclear antibody, type 1 [AGNA or SOX 1 antibody]), neuronal cytoplasm (Purkinje cell antibodies [PCA, types 1 (anti Yo), 2 and -Tr], collapsin response–mediator protein [CRMP] 5-IgG and amphiphysin-IgG), or to hippocampal and basal ganglionic synapses.

IgGs targeting specific neurotransmitter receptors in hippocampal synapses (NMDA [GluN1], AMPA [GluA1 and GluA2] and GABA-B) were sought by indirect immunofluorescence on HEK293 cells transfected with appropriate cDNAs (Euroimmun, Lübeck, Germany).

Antibodies reactive with neural cation channel complexes (neuronal voltage-gated calcium channels [VGCC, P/Q type and N type], voltage-gated potassium channel-complexes [VGKC-complexes], nicotinic acetylcholine receptors [AChR] of skeletal muscle-type [α1 subunit] and neuronal ganglionic-type [α3 subunit]) and glutamic acid decarboxylase 65 kDa isoform (GAD65) were detected by radioimmunoprecipitation assay. Skeletal muscle striational antibodies were detected by enzyme-linked immunosorbent assay. CRMP-5-IgG and amphiphysin-IgG were additionally sought by recombinant Western blot assays. Sera yielding positive results for VGKC–complex-IgG were tested further for IgG reactive with leucine-rich glioma-inactivated 1 (LGI1) protein or contactin-associated protein-2 (CASPR2) by a cell-based immunofluorescence assay (Euroimmun, Lübeck, Germany).

***Serological findings***

Six cases initially identified

Serums from 6 patients produced an identical nervous system-restricted synaptic pattern of immunoreactivity in several anatomical regions: cerebellum (molecular and granular layer), hippocampus, thalamus, cortex and myenteric nerves **(Supplementary figure 1A)**. Striatum was faintly immunoreactive, and kidney parenchyma was non-reactive. A130 kDa protein identified by western blot was confirmed to be contactin-1 by mass spectrometry after protein G magnetic bead capture from mouse cerebral lysate. The contactin-1 binding partner, CASPR1, was not detected.

Serum IgGs from all 6 patients, but no control human serum IgG, bound to recombinant full length contactin-1 protein on western blot. Contactin-1 seropositivity also was confirmed in all 6 by CBA, All patient serum IgGs bound to both contactin-1 expressing cells and cells co-expressing contactin-1 and CASPR1 (6), but not to cells expressing CASPR1 alone or mock transfected **(Supplementary figure 1B)**.

Six cases subsequently identified

Additionally, 5 patients were identified from our cohort of 233 cases (2%) with chronic/relapsing demyelinating neuropathy screened prospectively by cell-based assay. Of those 5 cases, 4 were positive by tissue IFA, 4 were positive by contactin-1expressing CBA, and 2 were positive by co-expressed contactin-1/CASPR1 CBA. No patient with monophasic AIDP or POEMS syndrome was seropositive.

Furthermore, 5 of 8 contactin-1 seropositive cases tested for IgG4 subtype were positive. None of the twelve cases were positive for neurofascin-155 IgG.

**References**

1. Gadoth A, Kryzer TJ, Fryer J, McKeon A, Lennon VA, Pittock SJ. Microtubule-associated protein 1B: Novel paraneoplastic biomarker. Ann Neurol 2017;81:266-277.

2. Honorat JA, Lopez-Chiriboga AS, Kryzer TJ, et al. Autoimmune gait disturbance accompanying adaptor protein-3B2-IgG. Neurology 2019;93:e954-e963.