THE LANCET Neurology

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lai M, Huijbers MGM, Lancaster E, et al. Investigation of Lgi1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 2010; published online June 28. DOI:10.1016/S1474-4422(10)70137-X.

Methods

Immunoprecipitation and immunoblot

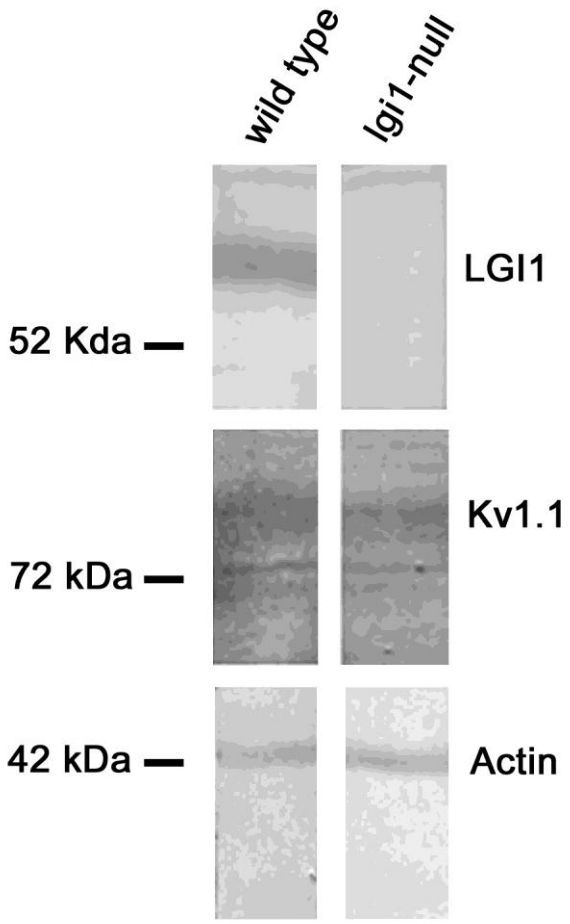
Live neurons obtained as above, were grown in 100mm wells (density 10^6 neurons/well), and incubated at 37°C with filtered patients' serum (diluted 1:500) for 1 hour. Neurons were then washed with PBS, lysed with buffer (NaCl 150mM, EDTA 1mM, tris(hydroxymethyl)aminomethane [Tris]-HCl 100mM, deoxycholate acid 0.5%, 1% Triton X-100 [Sigma Labs, St. Louis, MO], pH 7.5) containing protease inhibitors (P8340; Sigma Labs), and centrifuged at 16.1 x 10^3 *g* for 20 minutes at 4°C. The supernatant was retained and incubated with protein A/G agarose beads (20423; Pierce, Rockford, IL) overnight at 4°C, centrifuged, and the pellet containing the beads with patients' antibodies bound to the target cell surface antigen was then washed with PBS, aliquoted, and kept at -80°C. An aliquot of this pellet was resuspended in Laemmli buffer, boiled for 10 minutes, separated in a 4 to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the proteins visualized with EZBlue gel staining (G1041; Sigma Labs). Distinctive protein bands precipitated by patients' serum were excised from the gel and analyzed using mass spectrometry at the proteomic facility at the University of Pennsylvania. After characterization of the antigen, frozen aliquots of the indicated pellets were separated in a sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to nitrocellulose (162-0115; Bio-Rad, Hercules, CA), and probed with the indicated LGI1 antibody (made in rabbit, dilution 1:500; Ab30868, Abcam, Cambridge, MA). The reactivity was developed using the appropriate biotinylated secondary antibodies (1:2000) and the avidin-biotin peroxidase, diaminobenzidine method.

Mass spectrometry

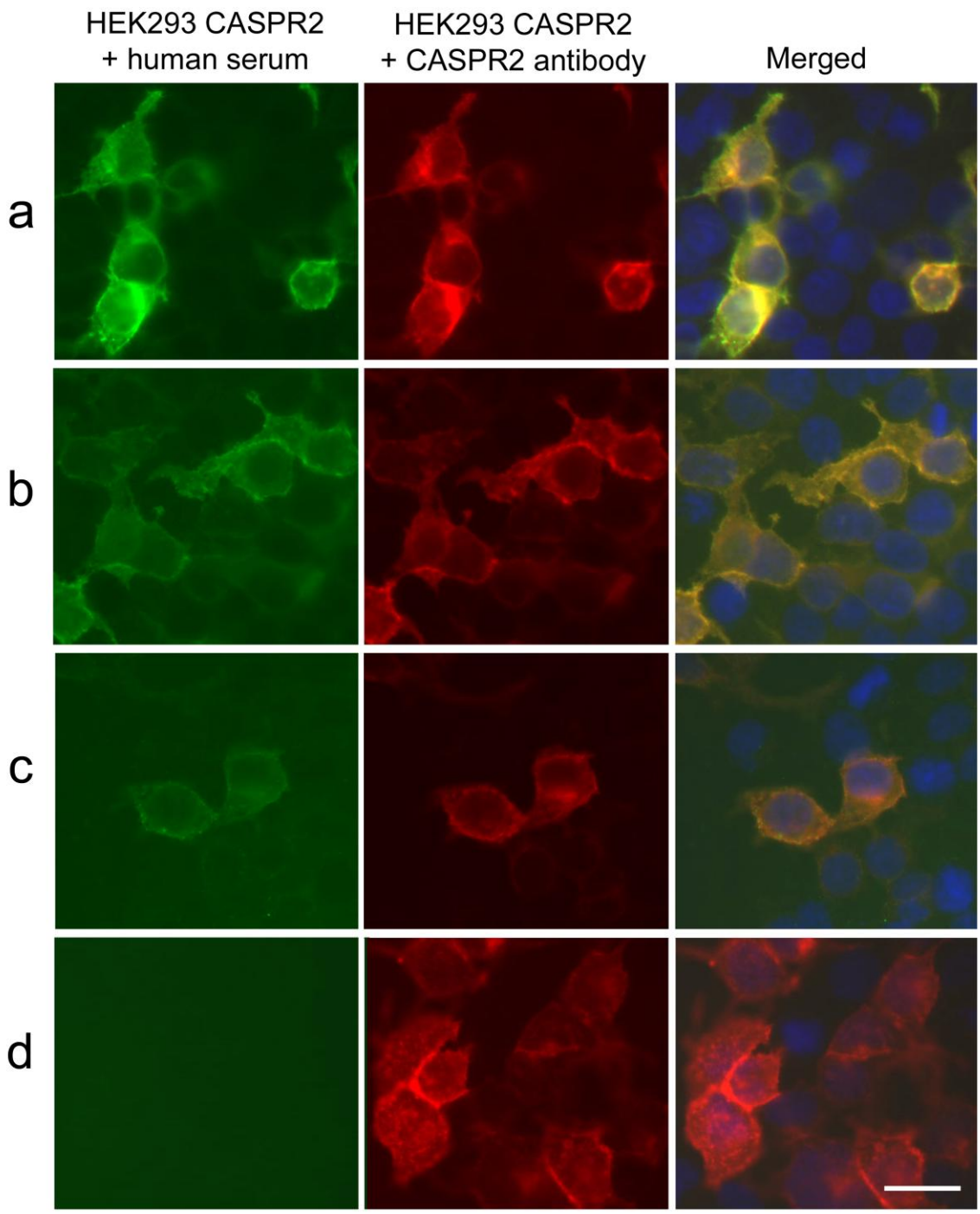
Protein bands from the gels were cut and sent for mass spectrometry to the Proteomics Core Facility of the Genomics Institute at the Abramson Cancer Center (University of Pennsylvania). Protein bands were trypsin digested and analyzed with a nano liquid chromatography (nano LC)/nanospray/linear ion trap (LTQ) mass spectrometer (Thermo Electron Corporation, San Jose, CA) as reported.[1] Briefly, 3 μl trypsin digested sample was injected with autosampler from Eksigent (Dublin, CA). The digested samples were separated on a 10 cm C18 column, using nano LC from Eksigent with 200 μl/minute flow rate, 45 minute gradient. Online nanospray was used to spray the separated peptides into LTQ, and Xcalibur software (Thermo Scientific, Waltham, MA) was utilized to acquire the raw data. The raw data files were searched using Mascot (Matrix Science, Boston, MA) against the NCBI and Swissprot databases (Swiss Institute of Bioinformatics (Basel, Switzerland). The cutoff for confident protein identification was $\geq$70.

Reference

1. Strader MB, Tabb DL, Hervey WJ, Pan C, Hurst GB. Efficient and specific trypsin digestion of microgram to nanogram quantities of proteins in organic-aqueous solvent systems. *Anal Chem* 2006; **78:** 125–34.



Supplemental Figure 1: **Expression of Kv1.1 by brain of *Lgi1*-null mice**
Immunoblots of hippocampal protein extracts (50 µg/lane) from brain of wild-type and *Lgi1*-null mice probed with a rabbit antibody against LGI1 (dilution 1:500, top lanes), a mouse monoclonal antibody against Kv1.1 (middle lanes), and a mouse monoclonal antibody against Actin (lower lanes, loading control; antibody obtained from Sigma, AC-40, dilution 1:200). The expression of Kv1.1 is preserved in *Lgi1*-null mice.



Supplemental Figure 2: **Patients' sera positive by ^{125}I-α-dendrotoxin RIA, but without LGI1 antibodies, react with CASPR2**

HEK293 cells expressing CASPR2 immunostained with human sera (left column) from a patient with severe encephalitis and seizures (a), a patient with neuromyotonia (b), a patient with Morvan's syndrome (c), and a normal individual (d). The middle column shows the corresponding reactivity with a rabbit antibody against CASPR2, and the last column shows the merged reactivities. All 3 patients' sera, but not the control, react with cells expressing CASPR2.Scale bar 20µm.

^{125}I-α-dendrotoxin RIA is a radioimmunoassay based on the immunoprecipitation of protein complexes containing VGKC labeled with ^{125}I-α-dendrotoxin.