

Supplementary Methods

All patients were tested for paraneoplastic autoantibodies (including anti-neuronal nuclear antibody type 1 [ANNA1; aka anti-Hu] and Collapsin response-mediator protein-5 [CRMP5]) as a part of routine clinical diagnostic testing in a Clinical Laboratory Improvement Amendment (CLIA) certified laboratory. Purkinje-cell cytoplasmic type 2 (PCA2) or microtubule-associated protein (MAP) 1B-IgG seropositivity was determined on the basis of characteristic tissue immunofluorescence and common a ~320 kD IgG band on western blot analysis of rat cerebellar and cortical extracts. All PCA2-IgG samples were also tested and confirmed positive on MAP1B fragment western blot. As the part the clinical testing validation pre-absorption study were performed.(1) Antigen specificities for CRMP5 and ANNA1 were confirmed by Western blot using recombinant human CRMP5 protein and native rat cerebellar Hu proteins respectively.

Indirect immunofluorescence assay (IFA)

IFA is a screening assay for all samples requested for paraneoplastic panel testing. Cryosection composed of adult mouse tissues including cerebral cortex, hippocampus, midbrain, cerebellum, gut, and kidney were tested with patient CSF, serum, and commercial antibodies. Tissue slides were fixed with 4% paraformaldehyde for 1 minute, 0.5% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) in phosphate buffered saline (PBS) was applied for permeabilization for a minute. Then blocked with 10% normal goat serum in PBS for one hour and washed with PBS. The patient serum was pre-absorbed with bovine liver powder at 1:240 dilution and CSF was non-absorbed 1:2 dilution. Slide was rinse with PBS for 3 times, then applied patient

samples and waiting for 40 minutes. After washing with PBS for 3 times, applied secondary antibody counterstained by FITC (fluorescein isothiocyanate) 1:100 (Southern Biotechnology Associates, Inc, Birmingham, AL, USA) on the slide. Mounted onto glass slides using ProLong Gold anti-fade medium (containing DAPI; Molecular Probes Thermo Fisher Scientific, USA). The specimens were observed using Olympus BX51 polarizing microscope with Olympus DP73 high-performance Peltier-cooled, 17.28 megapixel camera. The specimens identified as positive were titrated for the endpoint titer.

Native Neural Protein Western Blot

Western blot probing of rat cortex and cerebellar proteins with patients' IgGs revealed a common immunoreactive band at ~320 kDa; control human IgGs were non-reactive.

Western blot with MAP1B fragment 1 (Amino Acids Encompassed: 1–666) for confirmation

MAP1B coding region was amplified as 5 individual fragments overlapping by approximately 60 to 70 residues. IgG from all 40 patient's sera or CSF bound to recombinant MAP1B fragment (Amino Acids Encompassed: 1–666).(1)

Supplementary figure

Flowsheet demonstrating patient identification based on MAP1B-IgG seropositivity with/without other onconeural antibodies and available clinical information.

Abbreviation: ANNA1; anti-nuclear neuronal antibody-1, CRMP5 = collapsin response-mediator protein-5; IgG; immunoglobulin G, MAP1B; Microtubule-Associated Protein 1B

Reference

1. Gadoth A, Kryzer TJ, Fryer J, McKeon A, Lennon VA, Pittock SJ. Microtubule-associated protein 1B: Novel paraneoplastic biomarker. *Ann Neurol*. 2017;81(2):266–77.