Supplementary data 1

A. ELISA

A. Anti-neurofascin 155 antibodies  B. Anti-contactin 1 antibodies

corrected OD value  corrected OD value

0.0  0.5  1.0  1.5

131 patients with CIDP

B. Western blot analysis

220  120  100 (kDa)

Lane  1  2  3  4  5  6  7  8  9  10  11
Pt.  H  P1  P2  P3  P4  P5  P6  P7  P8  P9  N

OD by ELISA  0.005  0.702  0.795  0.421  0.867  0.812  1.356  0.39  0.748  0.819  0

H = Healthy subject
P = ELISA-positive patient
N = ELISA-negative patient
**Determination of anti-neurofascin 155 and anti-contactin 1 antibodies.**

(A) Reactivity to recombinant human neurofascin protein, which corresponds to neurofascin 155 (R&D Systems, Minneapolis, USA), or recombinant human contactin 1 protein (Sino Biological Inc., Beijing, China), was examined. Briefly, 96-well polystyrene enzyme-linked immunosorbent assay (ELISA) plates (Corning Life Sciences, Lowell, USA) were coated overnight with recombinant neurofascin protein, contactin 1 protein, or phosphate buffered saline as a control. After blocking, patient serum diluted at 1:200 with 1% bovine serum albumin in phosphate buffered saline was added as the primary antibody and incubated for 120 min. After three washes, horseradish peroxidase-conjugated anti-human IgG-Fc antibody (MP Biomedicals, Solon, USA) diluted at 1:500 with 1% bovine serum albumin in phosphate buffered saline was added as the secondary antibody and incubated for 90 min. The wells were washed again, and the chromogenic reaction with O-phenylenediamine in phosphate-citrate buffer was developed. The optical density (OD) values were measured at 490 nm (Bio-Rad Laboratories Inc., Hercules, USA). The corrected OD was calculated in duplicate by subtracting the OD values of phosphate buffered saline as the control OD. Among 131 patients with chronic inflammatory demyelinating polyneuropathy (CIDP), nine patients (7%) were positive for anti-neurofascin 155 antibodies (corrected OD ranged from 0.390 to 1.358) and one (1%) was positive for anti-contactin 1 antibodies (open circles), which were clearly separable from those of other CIDP patients (closed circles). Serum samples from 99 disease controls consisting of 57 patients with Guillain-Barré syndrome, 26 with Fisher syndrome, and 16 with multiple sclerosis, as well as 20 healthy subjects, were also investigated for anti-neurofascin 155 antibodies and showed no reactivity. For the anti-contactin 1
antibodies, serum samples from 83 disease controls consisting of 57 patients with Guillain-Barré syndrome and 26 with Fisher syndrome, as well as 20 healthy subjects, also showed no reactivity.

(B) Western blotting was performed on sera from nine patients found to be positive for the anti-neurofascin 155 antibodies by ELISA. Recombinant human neurofascin protein was mixed with sample buffer and then boiled for five minutes. Each antigen solution was loaded (250 ng/well) and then sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. After SDS-PAGE, proteins on the gel were electrophoretically transferred to polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany). After blocking, the polyvinylidene difluoride membrane was incubated overnight in patient serum diluted at 1:200 with blocking buffer at 4 ºC. The membrane was washed three times and incubated for one hour with horseradish peroxidase-conjugated anti-human IgG-Fc antibody (MP Biomedicals, Solon, USA), diluted at 1:5000 with blocking buffer. The membrane was washed again, and the band pattern (at approximately 120-140 kDa in reducing conditions) was revealed with LAS3000 (Fujifilm, Tokyo, Japan) using enhanced chemiluminescence. All nine patients showed a specific protein band pattern at approximately 120-140 kDa (P1-P9, lanes 2 to 10), whereas the healthy subjects (H, lane 1) and a CIDP patient with a negative ELISA result (N, lane 11) showed no reactivity.

The positive results of patients with anti-neurofascin 155 antibodies were also verified by cell-based assay. In brief, neurofascin 155-turbo GFP-transfected and naive HEK293 cells were evenly mixed. Serum samples (2.5 µl) were added to 47.5 µl of cell-containing solution (1:20 dilution). After incubation at 4°C for 60 min, cells were
washed and bound IgG was detected with Alexa 647-labeled anti-human IgG antibodies (Life Technologies, Carlsbad, CA), diluted 1:500. After incubation at 4°C for 60 min, cells were washed and analyzed by MACSQuant Analyzer (Miltenyi Biotec, Bergisch Gladbach, Germany). The mean fluorescence intensity (MFI) of cell-associated turbo GFP and Alexa 647 was measured for each sample. The MFI ratio was calculated by dividing Alexa 647 MFI of NF155-transfected cells by Alexa 647 MFI of neurofascin 155-untransfected cells, and delta MFI was calculated by subtracting Alexa-647 MFI of neurofascin 155-untransfected cells from Alexa 647 MFI of neurofascin 155-transfected cells.