Characterization of IgG4 anti-neurofascin 155 antibody-positive polyneuropathy

緒方, 英紀

https://doi.org/10.15017/1654703

出版情報: 九州大学, 2015, 博士（医学）, 課程博士
バージョン: published
権利関係: 全文ファイル公表済
Characterization of IgG4 anti-neurofascin 155 antibody-positive polyneuropathy

Hidenori Ogata, Ryo Yamasaki, Akio Hiwatashi, Nobuyuki Oka, Nobutoshi Kawamura, Dai Matsuse, Moji Kuwahara, Hidekazu Suzuki, Susumu Kusunoki, Yuichi Fujimoto, Koji Ikezoe, Hitaru Kishida, Fumiaki Tanaka, Takuya Matsushita, Hiroyuki Murai, and Jun-ichi Kira

1Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
2Department of Neurological Therapeutics, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
4Department of Neurology and Rehabilitation, National Hospital Organization Minami-Kyoto Hospital, Joyo, Japan
5Department of Neurology, Kawamura Hospital, Gifu, Japan
6Department of Neurology, Kinki University Faculty of Medicine, Osaka, Japan
7Department of Neurology, Matsuyama Red Cross Hospital, Matsuyama, Japan
8Department of Neurology, Yokohama City University Medical Center, Yokohama, Japan
9Department of Neurology and Stroke Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan

The positivity rate for anti-NF155 antibodies in CIDP patients was 18% (9/50), who all showed a predominance of IgG4 subclass. No other subjects were positive, except one GBS patient harboring IgG1 anti-NF155 antibodies. No anti-NF155 antibody carriers had anti-NF186 antibodies. Anti-NF155 antibody-positive CIDP patients had a significantly younger onset age, higher frequency of drop foot, gait disturbance, tremor and distal acquired demyelinating symmetric phenotype, greater cervical root diameter on magnetic resonance imaging neurography, higher cerebrospinal fluid protein levels, and longer distal and F-wave latencies than anti-NF155 antibody-negative patients. Marked symmetric hypertrophy of cervical and lumbosacral roots/plexuses was present in all anti-NF155 antibody-positive CIDP patients examined by neurography. Biopsied sural nerves from two patients with anti-NF155 antibodies demonstrated subperineurial edema and occasional paranodal demyelination, but no vasculitis, inflammatory cell infiltrates, or onion bulbs. Among anti-NF155 antibody-negative patients, treatment responders more frequently had daily oral corticosteroids and/or immunosuppressants in addition to intravenous immunoglobulins than nonresponders did. Interpretation: Anti-NF155 antibodies occur in a subset of CIDP patients with distal-dominant involvement and symmetric nerve hypertrophy.

Abstract

Objective: To investigate anti-neurofascin 155 (NF155) antibody-positive chronic inflammatory demyelinating polyneuropathy (CIDP). Methods: Sera from 50 consecutive CIDP patients diagnosed in our clinic, 32 patients with multiple sclerosis, 40 patients with other neuropathies including 26 with Guillain–Barré syndrome (GBS)/Fisher syndrome, and 30 healthy controls were measured for anti-NF antibodies by flow cytometry using HEK293 cell lines stably expressing human NF155 or NF186. Four additional CIDP patients with anti-NF155 antibodies referred from other clinics were enrolled for clinical characterization. Results: The positivity rate for anti-NF155 antibodies in CIDP patients was 18% (9/50), who all showed a predominance of IgG4 subclass. No other subjects were positive, except one GBS patient harboring IgG1 anti-NF155 antibodies. No anti-NF155 antibody carriers had anti-NF186 antibodies. Anti-NF155 antibody-positive CIDP patients had a significantly younger onset age, higher frequency of drop foot, gait disturbance, tremor and distal acquired demyelinating symmetric phenotype, greater cervical root diameter on magnetic resonance imaging neurography, higher cerebrospinal fluid protein levels, and longer distal and F-wave latencies than anti-NF155 antibody-negative patients. Marked symmetric hypertrophy of cervical and lumbosacral roots/plexuses were present in all anti-NF155 antibody-positive CIDP patients examined by neurography. Biopsied sural nerves from two patients with anti-NF155 antibodies demonstrated subperineurial edema and occasional paranodal demyelination, but no vasculitis, inflammatory cell infiltrates, or onion bulbs. Among anti-NF155 antibody-negative patients, treatment responders more frequently had daily oral corticosteroids and/or immunosuppressants in addition to intravenous immunoglobulins than nonresponders did. Interpretation: Anti-NF155 antibodies occur in a subset of CIDP patients with distal-dominant involvement and symmetric nerve hypertrophy.
Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) presents with various features in addition to demyelination, including muscle atrophy, sensory ataxia, tremor, asymmetrical or focal involvement, and nerve hypertrophy. However, biomarkers for these features remain ill defined. T-cell- and macrophage-mediated demyelination is assumed to play a major role in CIDP although some patients harbor autoantibodies against proteins at the nodes of Ranvier. Antibodies against contactin-1, a paranodal axolemmal protein, were detected in 6% of CIDP patients, who commonly showed advanced age, predominant motor involvement, aggressive symptom onset, and early axonal involvement. Another autoantigen, neurofascin (NF), comprised two major isoforms: axonal NF186, which interacts with neuronal cell adhesion molecules to cluster sodium channels at the nodes, and glial NF155, expressed at the paranodal loops of oligodendrocytes in the central nervous system and in Schwann cells of the peripheral nervous system, which interacts with axonal contactin-1 and contactin-associated protein (Caspr) to form a septal barrier excluding the nodal complex from the internodes. Contradictory results were reported for the presence of anti-NF186 antibodies in CIDP. One study showed a 12% positivity rate, and another 0%. Measurement of anti-NF155 antibodies by enzyme-linked immunoassortent assay in two studies revealed low positivity rates (2.5% and 3.8%) to human NF155 although 22% positivity to rat NF155 was reported. We previously reported that patients with combined central and peripheral demyelination (CCPD) frequently harbored anti-rat NF155 antibodies while some CIDP patients were also positive. In the present study, we developed a more specific antibody assay using human NF155 and found anti-NF155 antibodies occurred in a subset of CIDP patients with distal-dominant involvement and symmetric nerve hypertrophy.

Subjects and Methods

Subjects

Fifty-five consecutive CIDP patients, who met the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) definite electrodiagnostic criteria for CIDP, were diagnosed at Kyushu University Hospital between 2004 and 2014. Among them, 50 patients were enrolled in the present study, after excluding five patients whose sera were unavailable. Sera from 32 patients with multiple sclerosis (MS) according to the revised McDonald criteria. 26 patients with Guillain–Barré syndrome (GBS) or Fisher syndrome (FS), seven patients with vasculitic neuropathy, three patients with polynuropathy, organomegaly, endocrinopathy, M protein, and skin changes (POEMS) syndrome, three patients with hereditary motor and sensory neuropathy including two with the duplication of the peripheral myelin protein 22 gene, one patient with anti-myelin-associated glycoprotein antibody-positive neuropathy, and 30 healthy controls (HCs) were used for anti-NF155 and -NF186 antibody measurement. To characterize the clinical features, four additional anti-NF155 antibody-positive CIDP patients referred from other clinics were included. CIDP patients were classified into subtypes according to the EFNS/PNS CIDP guidelines: distal acquired demyelinating symmetric (DADS) neuropathy was diagnosed by the presence of disproportionately prolonged motor distal latencies (DL) resulting in a terminal latency index (TLI) ≤0.25 in at least two nerves; and responders to treatment were defined as patients whose Hughes functional scale scores at the last visit were decreased by ≥1 grade compared with those at peak of illness. This study was approved by the Kyushu University Hospital Ethics Committee.

Flow cytometric assay for anti-NF155 and anti-NF186 antibodies

Flow cytometry (FCM) detected IgG binding to cells expressing recombinant human NF155 or NF186 to exclude bias among evaluators. Generation of transformed cell lines stably expressing NF155 or NF186 is described in Data S1. NF155-turbo green fluorescent protein (turbo GFP)-transfected and naive HEK293 cells were evenly mixed and resuspended in Dulbecco’s modified Eagle’s medium containing 1% fetal bovine serum and 1 mmol/L ethylenediaminetetraacetic acid (EDTA) (FCM buffer) at a concentration of 1.0 × 10^6 cells/mL, and rotated at 4°C for 60 min. Serum samples (2.5 μL) were mixed with 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 in FCM buffer. After incubation at 4°C for 60 min, cells were washed and resuspended in 100 μL phosphate-buffered saline (PBS) containing 5 mmol/L EDTA 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. Cells expressing NF155-turbo GFP and naive cells were easily separable according to the MFI of turbo GFP (Fig. 1A). The MFI of cell-associated Alexa 647 was measured to detect human IgG bound to NF155, using cells without NF155 expression as a negative control. Positive and negative patients were clearly separable, and
MFI ratio and delta MFI values were proportionately decreased on sequential dilution of serum from an anti-NF155 antibody-positive patient (Fig. 1B). For each serum sample, the MFI ratio was calculated by dividing Alexa 647 MFI of NF155-transfected cells by Alexa 647 MFI of NF155-untransfected cells, and delta MFI was calculated by subtracting Alexa-647 MFI of NF155-untransfected cells from Alexa 647 MFI of NF155-transfected cells. Cutoff points for MFI ratio and delta MFI were set at 10 and 100, respectively, based on preliminary experiments. Antibodies against human recombinant NF186 protein were also measured using the same method. In anti-NF155 antibody-positive patients, IgG subclass profiles were examined using phycoerythrin (PE)-conjugated mouse anti-human IgG1, IgG2, IgG3, and IgG4 antibodies (Beckman Coulter Inc., Brea, CA) at 1:500 dilution. Conventional cell-based assays for anti-NF155 and anti-NF186 antibodies are described in Data S1.

**Immunostaining of mouse teased sciatic nerve fibers**

Sciatic nerves were dissected from 10-week-old C57BL/6 mice at our animal facility and fixed for 10 min in freshly prepared PBS containing 4% paraformaldehyde. After washing with PBS, fixed nerves were teased and transferred to glass slides, permeabilized with 2% Triton X-100 in PBS for 30 min, blocked in 10% goat serum and 1% Triton X-100 in PBS for 60 min, then incubated at 4°C in blocking solution containing rabbit anti-Caspr antibodies (diluted 1:500) (Abcam, Cambridge, U.K.) and sera from anti-NF155 antibody-positive CIDP patients or HCs (diluted 1:20). After 2 days, teased nerve fibers were washed three times in PBS for 30 min and incubated for 60 min with Alexa 488-labeled anti-human IgG and Alexa 647-labeled anti-rabbit IgG (Life Technologies), diluted 1:500. Finally, teased nerve fibers were washed three times in PBS for 30 min, mounted with PermaFluor (Thermo Scientific, Waltham, MA) and examined by confocal microscopy (A1; Nikon, Tokyo, Japan), using 488- and 638-nm lasers for excitation.

**Neuroimaging**

Magnetic resonance imaging (MRI) neurography with three-dimensional nerve-SHeath signal increased with INKed rest-tissue rapid acquisition with relaxation Enhancement Imaging (3D SHINKEI)\(^{18}\) of cervical roots and brachial plexuses, and lumbosacral roots and plexuses were acquired using a 3.0-T whole-body clinical imager (Achieva; Philips Healthcare, Best, the Netherlands) in all CIDP cases visiting Kyushu University Hospital after 2012 when 3D SHINKEI imaging became available, including seven anti-NF155 antibody-positive and 20 anti-NF155 antibody-negative patients. Details of 3D SHINKEI parameters are described in Data S1. Data analyses were performed by one neuroradiologist (A.H.), blinded to the diagnosis. The diameter of the cervical nerve root was defined as the maximum vertical length of the root. The largest root diameters among bilateral C5–C8 roots were compared between CIDP patients with and without anti-NF155 antibodies.

**Electrophysiology**

Nerve conduction studies were performed using conventional procedures as described previously.\(^{19}\) Motor nerve conduction studies including F-wave analyses were performed in the median, ulnar, tibial, and peroneal nerves. Sensory nerve conduction studies were performed in median, ulnar, and sural nerves. Bilateral values were used when available. Base-to-peak amplitudes were measured for compound muscle action potentials (CMAP) and sensory nerve action potentials (SNAP). TLI was calculated using the formula: distal conduction distance (mm)/forearm conduction velocity (m/sec)/DL (msec).

**Pathological studies of biopsied sural nerves**

Pathology of biopsied sural nerve specimens from two anti-NF155 antibody-positive CIDP patients was studied. Duration from onset to time of biopsy was 9 months in a 39-year-old female and 18 years in a 40-year-old male. SNAP of the sural nerve at the time of biopsy were absent in both patients. Nerves were assessed using standard Epon-embedded sections stained with toluidine blue, as described previously.\(^{20}\) Detailed methods are described in Data S1.

**Statistics**

For comparisons between two groups, qualitative variables were analyzed using the Fisher exact test. Continuous variables that followed a parametric distribution were analyzed by Student’s \(t\)-test, and nonparametric variables were analyzed by Mann–Whitney \(U\) test. Correlations were calculated with Pearson’s coefficient. The threshold for significance was set at \(P < 0.05\).

**Results**

**Frequency of anti-NF155 and anti-NF186 antibodies**

Positivity rates for anti-NF155 antibodies by FCM among patients with CIDP, MS, other neuropathies, or HCs were
Anti-NF155 Antibodies in CIDP

A. Scatter plot showing MFI ratios for Serum free, Negative case, and Positive case.

B. Graph showing delta MFI against dilution.

C. Immunofluorescence images of Human-NF155 expressing cells and Human-NF186 expressing cells.

D. Immunofluorescence images of Anti-NF155 antibody-negative healthy control and Anti-NF155 antibody-positive case.

E. Bar graph comparing delta MFI for Anti-NF155 antibodies.

F. Bar graph comparing delta MFI for Anti-NF186 antibodies.

G. 3D bar graph showing delta MFI for various Positive CIDP cases and HCs.
18% (9/50), 0% (0/32), 2.5% (1/40), and 0% (0/30), respectively, (Fig. 1E and S1A). Positive samples were also confirmed positive by conventional cell-based immunohistochemistry assays (Fig. 1C). Anti-NF155 antibody-positive CIDP patient sera bound to the paranodal regions of mouse teased sciatric nerve fibers, with a staining pattern similar to anti-Caspr antibody (Fig. 1D). When CIDP patients were classified into subtypes,\textsuperscript{15,16} DADS neuropathy had the highest positivity rate (3/5, 60.0%), while 6/36 (16.7%) typical CIDP patients were positive (Table S1). By contrast, no patients with multifocal acquired demyelinating sensory and motor neuropathy (MADSAM), focal, pure motor, or pure sensory types were positive for anti-NF155 antibodies. No subjects examined were positive by anti-NF186 antibody assay, except for one MS patient with weak positivity only observed by the MFI ratio (Fig. 1F and S1B).

**IgG subclass of anti-NF155 antibodies**

In all 13 anti-NF155 antibody-positive CIDP patients, including those from other clinics, IgG4 antibodies were predominant, while lower levels of IgG1 and IgG2 anti-NF155 antibodies were also present (Fig. 1G and S1C). One GBS patient with anti-NF155 antibodies harbored low IgG1 levels.

**Clinical features**

Age at onset was lower in anti-NF155 antibody-positive CIDP patients than in anti-NF155 antibody-negative patients ($P < 0.0001$) (Table 1). DADS phenotype, drop foot, gait disturbance, and tremor were more frequently observed in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients ($P = 0.0014$, 0.0242, 0.0484, and 0.0300, respectively). Cerebrospinal fluid (CSF) protein levels were markedly higher in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients ($P < 0.0001$). The frequency of brain MRI lesions (suggestive of inflammatory demyelination) was threefold greater in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients. When only CIDP patients subjected to MRI neurography in our department were analyzed, similar trends were observed for anti-NF155 antibody status (Table S2).

**MRI neurography findings**

The cervical and lumbosacral roots/plexuses of all seven anti-NF155 antibody-positive CIDP patients showed marked symmetric hypertrophy (Fig. 2A and B). Measurement of the largest root diameters among bilateral C5–C8 roots were significantly greater in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (7.7 ± 1.3 mm vs. 4.9 ± 2.0 mm, $P = 0.0020$, Fig. 2C). The frequency of the largest nerve roots with a diameter >6.0 mm was more common in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (100% vs. 25%, $P = 0.0009$). The root diameters showed a trend toward positive correlation with disease duration in anti-NF155 antibody-positive CIDP patients ($r = 0.739, P = 0.0578$, Fig. 2D), but not in anti-NF155 antibody-negative patients (data not shown).
Nerve conduction study findings

Significantly longer F-wave latencies of the median, ulnar, and tibial nerves ($P = 0.0033$, $<0.0001$, and $0.0109$, respectively) and DL of the ulnar and tibial nerves ($P = 0.0009$ and $0.0001$, respectively) were observed in anti-NF155 antibody-positive CIDP patients than in anti-NF155 antibody-negative CIDP patients (Table 2). Although DL in the median nerve did not differ significantly between the two groups, the...
frequency of median nerves with >50% DL prolongation above the upper normal limit (an EFNS/PNS electrodiagnostic criterion) was higher in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (81.0% vs. 43.7%, \( P = 0.0029 \)). CMAP amplitudes and TLIs of the tibial
nerve were lower in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients ($P = 0.0011$ and $<0.0001$, respectively). Motor conduction velocity did not differ significantly between groups. Sensory conduction velocity (SCV) of the median and ulnar nerves was significantly decreased in anti-NF155 antibody-positive patients compared with anti-NF155 antibody-negative patients ($P = 0.0038$ and $<0.0001$, respectively), and unevoked SNAP frequencies in the median and ulnar nerves were higher in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (81.0% vs. 42.9%, $P = 0.0026$ and 75.0% vs. 38.2%, $P = 0.049$, respectively). These significant differences remained after eliminating patients with MADSAM and focal phenotypes (data not shown).

Abnormalities in DL of the median, ulnar, and Tibial nerves, in F-wave latency of the ulnar and tibial nerves, in CMAP amplitudes of the tibial nerve, and in SCV of the median, ulnar, and sural nerves were significantly higher in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (Table S3).

**Pathological findings of biopsied sural nerves**

Subperineurial edema was evident and demyelinated fibers and naked axons were occasionally present in both specimens examined (Fig. 2E-J). Myelinated fiber loss was more evident in the patient with longer disease duration (4930/mm²) compared with shorter disease duration (2200/mm²) in the anti-NF155 antibody-negative patient. Improved myelination was evident in the patient with anti-NF155 antibody-negative (Fig. 2E). Neurons and endoneurial edema were not present in any of the sural nerve biopsies.

### Table 2. Nerve conduction study findings in CIDP patients with and without anti-NF155 antibodies.

<table>
<thead>
<tr>
<th>Objective</th>
<th>All CIDP patients</th>
<th>NF155 antibody-negative CIDP</th>
<th>NF155 antibody-positive CIDP</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median nerve</td>
<td>$N = 92$</td>
<td>$N = 71$</td>
<td>$N = 21$</td>
<td></td>
</tr>
<tr>
<td>Distal latency (msec)</td>
<td>$6.9 \pm 3.0$ ($92/92$)</td>
<td>$6.7 \pm 3.3$ ($71/71$)</td>
<td>$7.7 \pm 1.4$ ($21/21$)</td>
<td>NS</td>
</tr>
<tr>
<td>Terminal latency index</td>
<td>$0.36 \pm 0.18$</td>
<td>$0.37 \pm 0.19$</td>
<td>$0.32 \pm 0.16$</td>
<td>NS</td>
</tr>
<tr>
<td>MCV (m/sec)</td>
<td>$35.0 \pm 12.2$</td>
<td>$35.7 \pm 12.1$</td>
<td>$32.7 \pm 12.4$</td>
<td>NS</td>
</tr>
<tr>
<td>CMAP amplitude (mV)</td>
<td>$4.7 \pm 3.3$</td>
<td>$4.7 \pm 3.7$</td>
<td>$4.7 \pm 1.8$</td>
<td>NS</td>
</tr>
<tr>
<td>F-wave latency (msec)</td>
<td>$45.3 \pm 13.6$ ($62/91$)</td>
<td>$42.4 \pm 11.4$ ($46/70$)</td>
<td>$53.7 \pm 16.3$ ($16/21$)</td>
<td>0.0033</td>
</tr>
<tr>
<td>SCV (m/sec)</td>
<td>$43.8 \pm 9.7$ ($44/91$)</td>
<td>$45.1 \pm 8.8$ ($40/70$)</td>
<td>$30.8 \pm 10.1$ ($4/21$)</td>
<td>0.0038</td>
</tr>
<tr>
<td>SNAP amplitude ($\mu$V)</td>
<td>$5.1 \pm 3.7$</td>
<td>$5.3 \pm 3.7^2$</td>
<td>$3.2 \pm 2.9^2$</td>
<td>NS</td>
</tr>
<tr>
<td>Ulnar nerve</td>
<td>$N = 88$</td>
<td>$N = 68$</td>
<td>$N = 20$</td>
<td></td>
</tr>
<tr>
<td>Distal latency (msec)</td>
<td>$4.9 \pm 1.8$ ($88/88$)</td>
<td>$4.6 \pm 1.8$ ($68/68$)</td>
<td>$6.0 \pm 1.1$ ($20/20$)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Terminal latency index</td>
<td>$0.46 \pm 0.19$</td>
<td>$0.48 \pm 0.19^3$</td>
<td>$0.42 \pm 0.16$</td>
<td>NS</td>
</tr>
<tr>
<td>MCV (m/sec)</td>
<td>$37.6 \pm 13.5$</td>
<td>$39.0 \pm 13.0$</td>
<td>$32.9 \pm 14.3$</td>
<td>0.0758</td>
</tr>
<tr>
<td>CMAP amplitude (mV)</td>
<td>$4.2 \pm 2.7$</td>
<td>$4.1 \pm 2.9$</td>
<td>$4.3 \pm 2.0$</td>
<td>NS</td>
</tr>
<tr>
<td>F-wave latency (msec)</td>
<td>$44.5 \pm 17.0$ ($56/88$)</td>
<td>$37.8 \pm 8.4$ ($40/68$)</td>
<td>$61.4 \pm 21.2$ ($16/20$)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SCV (m/sec)</td>
<td>$43.2 \pm 9.0$ ($47/88$)</td>
<td>$44.9 \pm 7.5$ ($42/68$)</td>
<td>$28.8 \pm 7.6$ ($5/20$)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SNAP amplitude ($\mu$V)</td>
<td>$3.7 \pm 3.0$</td>
<td>$4.0 \pm 3.1^2$</td>
<td>$1.2 \pm 0.97^2$</td>
<td>0.0529</td>
</tr>
<tr>
<td>Tibial nerve</td>
<td>$N = 92$</td>
<td>$N = 71$</td>
<td>$N = 21$</td>
<td></td>
</tr>
<tr>
<td>Distal latency (msec)</td>
<td>$8.0 \pm 4.2$ ($75/92$)</td>
<td>$7.0 \pm 3.7$ ($60/71$)</td>
<td>$12.2 \pm 3.8$ ($15/21$)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Terminal latency index</td>
<td>$0.49 \pm 0.18$</td>
<td>$0.53 \pm 0.17$</td>
<td>$0.31 \pm 0.11$</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCV (m/sec)</td>
<td>$32.6 \pm 9.8$</td>
<td>$33.7 \pm 9.6$</td>
<td>$28.5 \pm 9.4$</td>
<td>0.0741</td>
</tr>
<tr>
<td>CMAP amplitude (mV)</td>
<td>$4.0 \pm 4.4$</td>
<td>$4.8 \pm 4.5$</td>
<td>$0.78 \pm 1.8$</td>
<td>0.0011</td>
</tr>
<tr>
<td>F-wave latency (msec)</td>
<td>$64.3 \pm 14.6$ ($44/92$)</td>
<td>$62.3 \pm 14.1$ ($39/71$)</td>
<td>$79.7 \pm 9.5$ ($5/21$)</td>
<td>0.0109</td>
</tr>
<tr>
<td>SCV (m/sec)</td>
<td>$44.5 \pm 6.1$ ($52/93$)</td>
<td>$45.2 \pm 6.4$ ($41/71$)</td>
<td>$41.9 \pm 3.9$ ($11/22$)</td>
<td>NS</td>
</tr>
<tr>
<td>SNAP amplitude ($\mu$V)</td>
<td>$7.0 \pm 5.8$</td>
<td>$6.6 \pm 5.8$</td>
<td>$8.4 \pm 5.9$</td>
<td>NS</td>
</tr>
</tbody>
</table>

CIDP. chronic inflammatory demyelinating polyneuropathy; CMAP, compound muscle action potentials; MCV, motor conduction velocity; $N$, number of examined nerves; NF, neurofascin; SCV, sensory conduction velocity; SNAP, sensory nerve action potentials; NS, not significant.

1All continuous values are shown as mean ± SD, with number of evoked nerves/number of examined nerves in parentheses. Normal values of distal latencies: median, 3.49 ± 0.34 m/sec; ulnar, 2.59 ± 0.39 m/sec; tibial 3.96 ± 1.00 m/sec. Normal values of MCV: median, 57.7 ± 4.9 m/sec; ulnar, 58.7 ± 5.1 m/sec; tibial, 48.5 ± 3.6 m/sec. Normal values of CMAP amplitudes: median, 7.0 ± 3.0 mV; ulnar, 5.7 ± 2.0 mV; tibial 5.8 ± 1.9 mV. Normal values of F-wave latencies: median, 26.2 ± 2.2 m/sec; ulnar, 27.6 ± 2.2 m/sec; tibial 47.7 ± 5.0 m/sec. Upper limit of normal (ULN) of distal latencies: median, 4.2 m/sec; ulnar, 3.4 m/sec; tibial 6.0 m/sec. Lower limit of normal (LLN) of MCV: median, 48 m/sec; ulnar, 49 m/sec; tibial, 41 m/sec. LLN of CMAP amplitudes: median, 3.5 mV; ulnar, 2.8 mV; tibial, 2.9 mV. ULN of F-wave latencies: median, 31 msec; ulnar, 32 msec; tibial, 58 msec. LLN of SCV: median, 44 m/sec; ulnar, 44 m/sec; sural, 45 m/sec.

2The unevoked SNAP frequencies in the median and ulnar nerves were significantly higher in anti-NF155 antibody-positive patients than in anti-NF155 antibody-negative patients (81.0% vs. 42.9%, $P = 0.0026$, 75.0% vs. 38.2%, $P = 0.049$, respectively).

3One outlier was omitted.
duration (6420/mm²), although fiber loss was not severe (control = 7710 ± 1130/mm²). Both specimens showed no vasculitis, infiltration of inflammatory cells, or onion bulb formation. The teased nerve fiber study demonstrated the presence of paranodal demyelination in 3/50 myelinated fibers in both patients.

Differences in adopted immunotherapies between treatment responders and nonresponders in anti-NF155 antibody-positive CIDP patients

Improvements in neurological findings of anti-NF155 antibody-positive CIDP patients after IVIg, intravenous corticosteroid pulse therapy, oral corticosteroids, and plasmapheresis were observed in 4/13 (30.8%), 3/8 (37.5%), 5/8 (62.5%), and 4/6 (66.7%) patients, respectively. Among 13 anti-NF155 antibody-positive CIDP patients, eight patients were treatment responders, while five were nonresponders. Comparison of treatment modalities between responders and nonresponders showed that the number of treatment modalities was greater in responders than in nonresponders (P = 0.0118) (Table 3). IVIg was administered to all patients regardless of treatment response, while corticosteroid therapy was used significantly more often in responders than in nonresponders. Daily oral corticosteroids and/or immunosuppressants were also administered to responders more frequently than nonresponders (P = 0.0047).

Discussion

The present study demonstrated anti-human NF155 antibodies were present in 18% of CIDP patients tested by specific cell-based FCM assays using human NF155 and that IgG4 anti-NF155 antibodies were associated with younger onset age, higher frequencies of drop foot, gait disturbance, tremor and DADS phenotype, greater spinal root diameter on MRI neurography, higher CSF protein levels, and more pronounced prolongation of distal and F-wave latencies in CIDP patients.

By using human recombinant NF155 and cell-based FCM assays, the specificity of our cell-based anti-NF155 antibody assay to CIDP was 98.9% based on a total IgG assay and 100% based on IgG4 subclass assay. Nonetheless, the positivity rate of anti-NF155 antibodies among CIDP patients in this study was much higher than in two previous studies (2.5%7 and 3.8% by ELISA 8) using human recombinant NF155 as an antigen. Although another study9 reported similar positivity rates to ours, this study performed ELISA using the extracellular domain of rat recombinant NF155 derived from NS0 murine myeloma cells, which may cause nonspecific binding as reported previously.7 The discrepancy between our study and two previous studies using the same human NF155 may be attributable in part to differences in inclusion criteria and races studied. We included only electrodiagnostic definite CIDP patients, who were classified to the definite CIDP category, whereas one article did not describe the diagnostic criteria used,7 and another adopted EFNS/PNS diagnostic criteria but did not state whether probable or possible CIDP cases were enrolled.8

A previous study described the features of four anti-NF155 antibody-positive patients only, where four cases presented with severe motor and sensory involvement, three cases with predominantly distal involvement, and three cases with disabling tremor.8 In the current study, anti-NF155 antibody was not detected in any patients.

### Table 3. Comparison of treatment modality between responders and nonresponders in anti-NF155 antibody-positive CIDP patients

<table>
<thead>
<tr>
<th></th>
<th>Total patients (N = 13)</th>
<th>Responders (N = 8)</th>
<th>Nonresponders (N = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of applied immunotherapies(^1) (median, IQR)</td>
<td>2 (2.3, 3.5)</td>
<td>3 (2.25, 4)</td>
<td>2 (1, 2)</td>
<td>0.0118</td>
</tr>
<tr>
<td>IVIg</td>
<td>13/13 (100)</td>
<td>8/8 (100)</td>
<td>5/5 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>10/13 (76.9)</td>
<td>8/8 (100)</td>
<td>2/5 (40.0)</td>
<td>0.0350</td>
</tr>
<tr>
<td>Oral corticosteroids</td>
<td>8/13 (61.5)</td>
<td>7/8 (87.5)</td>
<td>1/5 (20.0)</td>
<td>0.0319</td>
</tr>
<tr>
<td>Intravenous corticosteroids</td>
<td>8/13 (61.5)</td>
<td>7/8 (87.5)</td>
<td>1/5 (20.0)</td>
<td>0.0319</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>6/13 (46.2)</td>
<td>5/8 (62.5)</td>
<td>1/5 (20.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Other immunosuppressants</td>
<td>4/13 (30.8)</td>
<td>4/6 (66.7)</td>
<td>0/5 (0.0)</td>
<td>N/S</td>
</tr>
<tr>
<td>Corticosteroids and plasmapheresis</td>
<td>5/13 (38.5)</td>
<td>5/8 (62.5)</td>
<td>0/5 (0.0)</td>
<td>0.0754</td>
</tr>
<tr>
<td>Daily immunosuppressants and/or corticosteroids at the last visit</td>
<td>7/13 (53.8)</td>
<td>7/8 (87.5)</td>
<td>0/5 (0.0)</td>
<td>0.0047</td>
</tr>
</tbody>
</table>

NF, neurofascin; CIDP, chronic inflammatory demyelinating polyneuropathy; IVIg, intravenous immunoglobulin; IQR, interquartile range; n, number of involved patients; N, number of patients collated; NS, not significant.

\(^1\)Applied immunotherapies among IVIg, corticosteroids, plasmapheresis, and other immunosuppressants (azathioprine or cyclosporine) were counted from 0 to 4.
with MADSAM or focal CIDP. We revealed, for the first time, statistically significant differences in clinical features between anti-NF155 antibody-positive and -negative cases. Anti-NF155 antibody-positive cases had a younger age at disease onset, in contrast to older age at onset in anti-contactin-1 antibody-positive CIDP preferentially affecting axons in the early course.² Predominantly distal motor and sensory involvement and disabling tremor were significantly more common in anti-NF155 antibody-positive cases than in antibody-negative cases, in accord with a previous small case series.⁸ However, proximal parts of limbs can also be affected as shown in our and previous studies, suggesting the emergence of anti-NF155 antibodies are not confined to the DADS phenotype but might also be present in the typical CIDP phenotype. We previously reported the occurrence of anti-NF155 antibodies in CCPD patients.¹⁰ In the present study of CIDP patients, brain MRI lesions suggestive of demyelination were more common in anti-NF155 antibody-positive CIDP patients than in antibody-negative patients, suggesting an association of anti-NF155 antibodies with brain white matter involvement. Further large-scale studies are required to clarify this point.

A distinguishing feature of anti-NF155 antibody-positive CIDP is marked symmetric nerve hypertrophy on MRI. Nerve hypertrophy is regarded as a cardinal feature of CIDP by both pathological and neuroimaging studies;²¹,²²; however, relevant immunological biomarkers for hypertrophy are undefined. Here, the presence of anti-NF155 antibodies was associated with diffuse symmetric but not focal nerve hypertrophy. We examined all CIDP patients who regularly visited Kyushu University Hospital from 2012 when 3D SHINKEI imaging became available. Although CIDP cases examined before the introduction of 3D SHINKEI imaging and four anti-NF155 antibody-positive CIDP cases referred from other clinic were not examined for MRI neurography, we consider it relevant that all anti-NF155 antibody-positive cases examined by MRI neurography in our institute uniformly showed hypertrophy of nerve roots and proximal nerve segments. As root diameters in anti-NF155 antibody-positive cases showed a trend toward a positive correlation with disease duration, the presence of anti-NF155 antibodies may predict progressive nerve hypertrophy during the late course of disease.

Marked prolongation of distal and F-wave latencies in anti-NF155 antibody-positive CIDP patients suggested preferential involvement of nerve terminals and spinal roots. More frequent and severe involvement of median and ulnar nerves compared with sural nerves in sensory nerve conduction studies indicated that distal parts of nerves might be more vulnerable in anti-NF155 antibody-positive patients, because median and ulnar sensory conduction studies involve nerve terminals, while those of the sural nerve involve the intermediated nerve segment.²³ Extremely high CSF protein levels in anti-NF155 antibody-positive patients also indicated preferential involvement of spinal roots. Preferential sites of involvement in anti-NF155 antibody-positive CIDP are compatible with the observation that autoantibody-mediated neuropathies preferentially affect the distal nerve terminals and nerve roots where the blood–nerve barrier is anatomically deficient or leaky.²⁴,²⁵

Sera from anti-NF155 antibody-positive CIDP patients bound specifically to paranodal regions of peripheral nerves, suggesting the paranodes are primary targets. However, anti-NF155 antibodies observed in this study were mainly IgG4, which lacks complement binding and activating capabilities. Biopsied sural nerve specimens from two anti-NF155 antibody-positive CIDP patients showed no inflammatory features, although SNAP were not evoked in either patient. Collectively, these observations suggest the primary role of IgG4 anti-NF155 antibodies may be blockade of interactions between NF155 and Caspr/contactin-1, leading to conduction failure. This might be consistent with the finding that myelinating glia-specific ablation of NF155 induces a loss of septa-like transverse bands at the paranodal axoglial junction and decreased conduction velocities in peripheral nerves even though the thickness of myelin at the internodes was intact.²⁶ We assume that blockade of NF155 and Caspr/contactin-1 interactions may lead to features similar to those of myelinating-glial-specific NF155 null mice.

Because of a lack of histological examination of proximal nerve segments, the mechanism of marked symmetric spinal root and plexus hypertrophy in anti-NF155 antibody-positive CIDP cases remains to be elucidated. In anti-NF155 antibody-positive CIDP patients, nerve edema caused by severe disruption of the blood–nerve barrier at spinal roots, indicated by a marked increase in CSF protein levels, might be one mechanism underlying symmetric spinal root/plexus hypertrophy. Even the sural nerve showed subperineurial edema, supporting this mechanism. Alternatively, onion bulb formation might be partly responsible for nerve hypertrophy as shown in biopsied CIDP brachial plexus,²¹ although we did not observe such features in the biopsied sural nerves.

Anti-NF155 antibodies were previously found in a fraction of CIDP patients' refractory to IVIg.⁸ Our results also suggest that IVIg alone is not sufficient to improve the disabilities of such patients. However, our study had some limitations concerning the evaluation of treatment efficacy. First, the number of anti-NF155 antibody-positive patients was small. Second, we used the Hughes score, which may not be adequate for evaluating upper limb involvement. However, all our anti-NF155...
antibody-positive cases had gait disturbance as a cardinal feature. Thus, the Hughes score might be useful for evaluating overall effectiveness of treatments. Third, various treatment regimens were applied because the study was retrospective. With these limitations in mind, corticosteroids combined with IVIg might be more beneficial than IVIg alone, because corticosteroids were used more often in treatment responders than in nonresponders. Corticosteroids are widely used to treat diseases related to disease-specific IgG4 autoantibodies, such as pemphigus and thrombotic thrombocytopenic purpura. Of note, treatment responders were more frequently administered daily oral corticosteroids and/or immunosuppressants compared with nonresponders, suggesting the necessity of long-term immunosuppression for sustained improvement. Future prospective studies to clarify adequate treatment modalities in anti-NF155 antibody-positive CIDP are required.

In conclusion, anti-NF155 antibodies measured by high-specificity assays defined a distinct subset of CIDP patients presenting with younger onset age, tremor, extremely high CSF protein levels, symmetric spinal root and plexus hypertrophy, and marked prolongation of distal and F-wave latencies, and therefore might be a biomarker for a unique subset of CIDP.

Acknowledgments
This study was partly supported by Health and Labour Sciences Research Grants on Intractable Diseases (H24-Nanchitou-Ippan-055 and H26-Itaku-Nan-Ippan-069) from the Ministry of Health, Labour, and Welfare, Japan, KAKENHI (24591265) and a “Gial Assembly” Grant-in-Aid for Scientific Research on Innovative Areas (FY2013-2017) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Center for Clinical and Translational Research of Kyushu University Hospital. We thank M. Satake (Department of Neurology, Hamanomachi Hospital), K. Sonoda (Department of Neurology, Kagoshima Medical Association Hospital), T. Mukaino and K. Takase (Department of Neurology, Aso Iizuka Hospital), W. Shiraishi, Y. Iwasaka, and A. Yamamoto (Department of Neurology, Japan Community Health Care Organization Kyushu Hospital), and H. Kishikawa (Faculty of Medicine, Kyushu University) for their cooperation.

Author Contributions

Conflict of Interest

References
Anti-NF155 Antibodies in CIDP


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary methods.

Table S1. Frequency of anti-NF155 antibodies among patients with various neurological diseases and healthy controls.

Table S2. Demographic features of CIDP patients with and without anti-NF155 antibodies evaluated by 3D SHINKEI sequence®.

Table S3. Frequencies of abnormal recordings in CIDP patients with and without anti-NF155 antibodies.

Figure S1. MFI ratios for anti-NF155 and anti-NF186 antibodies.