Neurofascin-155 IgG4 in chronic inflammatory demyelinating polyneuropathy

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ABSTRACT

Objective: We report the clinical and serologic features of Japanese patients with chronic inflammatory demyelinating polyneuropathy (CIDP) displaying anti-neurofascin-155 (NF155) immunoglobulin G4 (IgG4) antibodies.

Methods: In sera from 533 patients with CIDP, anti-NF155 IgG4 antibodies were detected by ELISA. Binding of IgG antibodies to central and peripheral nerves was tested.

Results: Anti-NF155 IgG4 antibodies were identified in 38 patients (7%) with CIDP, but not in disease controls or normal participants. These patients were younger at onset as compared to 100 anti-NF155–negative patients with CIDP. Twenty-eight patients (74%) presented with sensory ataxia, 16 (42%) showed tremor, 5 (13%) presented with cerebellar ataxia associated with nystagmus, 3 (8%) had demyelinating lesions in the CNS, and 20 of 25 (80%) had poor response to IV immunoglobulin. The clinical features of the antibody-positive patients were statistically more frequent as compared to negative patients with CIDP (n = 100). Anti-NF155 IgG antibodies targeted similarly central and peripheral paranodes.

Conclusion: Anti-NF155 IgG4 antibodies were associated with a subgroup of patients with CIDP showing a younger age at onset, ataxia, tremor, CNS demyelination, and a poor response to IV immunoglobulin. The autoantibodies may serve as a biomarker to improve patients' diagnosis and guide treatments. *Neurology*® 2016;86:800–807

GLOSSARY

Caspr1 = contactin-associated protein-1; **CCPD** = combined central and peripheral demyelination; **CI** = confidence interval; **CIDP** = chronic inflammatory demyelinating polyneuropathy; **CNTN1** = contactin 1; **GBS** = Guillain-Barré syndrome; **IgG4** = immunoglobulin G4; **IVIg** = IV immunoglobulin; **MS** = multiple sclerosis; **NF155** = neurofascin-155; **OR** = odds ratio; **PNS** = peripheral nervous system.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most common acquired immune-mediated neuropathy worldwide and is clinically heterogeneous.[1] Proven treatments for CIDP include corticosteroids, plasma exchange, and IV immunoglobulin (IVIg). However, the response rates to treatments are highly heterogeneous between patients. This emphasizes that patients and clinicians require biomarkers to identify CIDP subgroups and guide specific immunotherapeutic options.

Immunoglobulin G4 (IgG4) autoantibodies to neurofascin-155 (NF155) were recently documented in patients with CIDP.[2,3] NF155 belongs to the L1 family of adhesion molecules and is expressed at paranodes by the terminal loops of myelin and associates with the axonal cell adhesion molecules CNTN1 and contactin-associated protein-1 (Caspr1).[4] This ternary complex of glycoproteins is required for the rapid propagation of the nerve impulses along myelinated axons.[5,6] Three of 4 patients with anti-NF155 IgG4 showed disabling tremor and all showed poor response to IVIg.[3] This suggested that IgG4 autoantibodies participate in CIDP pathogenesis; nonetheless, the low number of reactive patients precluded statistical correlation.

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Supplemental data at Neurology.org

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Anti-NF155 antibodies were also reported by a single Japanese group in a proportion of patients with combined central and peripheral demyelination (CCPD).[7,8] However, it is unclear whether these autoantibodies belong to the IgG4 subclass, and whether these CCPD cases are considered as definite CIDP cases with CNS involvement. To answer these questions, we have examined the clinical and serologic features of 38 Japanese patients with CIDP and anti-NF155 IgG4 antibodies including 3 patients with CCPD.

METHODS

Patients and sera. Serum samples from 533 patients with CIDP were obtained before immunotherapy and stored at −80°C until use. Besides, sera from 200 patients with Guillain-Barré syndrome (GBS) and 100 with multiple sclerosis (MS) were used as disease controls, and sera from 55 healthy controls were used. These control sera were obtained before immunotherapy. Cerebellar ataxia was defined as ataxia with dysarthria and nystagmus. Therefore, the combination of sensory and cerebellar ataxia was defined as deep sense impairment with the above cerebellar symptoms. Patients were considered responsive to the therapy if improvements in muscle weakness or sensory disturbances were observed within 4 weeks after the administration. IVIg treatment regimen was 400 mg/kg/d for 5 days, and the prednisolone regimens were 1 mg/kg/d or 1 g/d for 3 days. Combined treatment with corticosteroids and other immunosuppressive agents was used in some patients but not all.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained from each participant. Diagnoses of CIDP, GBS, and MS were made by primary clinicians based on published criteria,[9–11] and clinical information, progress upon discharge, and follow-up were obtained from each patient. The diagnosis of CIDP for patients associated with anti-NF155 IgG4, anti-CNTN1 IgG4, and neither (n = 100) was confirmed by one of the authors (Y.F.). The study was approved by the ethics committee of Dokkyo Medical University and National University of Singapore.

Constructs. All truncations were constructed from Myc-tagged human NF155 (NM_001160331.1) using the site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA).

Cell-binding assay. Detailed methods for cell-binding assay are described in e-Methods on the *Neurology*® Web site at Neurology.org.

ELISA. IgG, IgA, or IgM antibodies against NF155, NF186, and CNTN1 were tested as described elsewhere with minor modifications.[12] Fifty microliters per well of human recombinant proteins diluted in phosphate-buffered saline were coated onto microtiter plates (NF155 and NF186 [OriGene technologies], 1 μg/mL; CNTN1 [Sino Biological Inc.], 0.5 μg/mL) at 4°C for overnight. Wells were blocked with 0.5% casein sodium in phosphate-buffered saline containing 0.05% Tween 20 for 1 hour at 37°C. Serum was considered positive when the calculated optical density was 0.5 or higher at 1:500 dilution. A complement deposition assay using NF155 as antigens was performed as described previously.

Biotinylated anti-human IgG subclass-specific antibodies (1 mg/mL) (IgG1, clone 8c/6-39; IgG2, clone HP6014; IgG3, clone HP6050; and IgG4, clone HP6025) were purchased from Sigma-Aldrich (St. Louis, MO). The specificity of each antibody was confirmed by ELISA against purified human IgG1, IgG2, IgG4 (Athens Research and Technology), or IgG3 (Sigma-Aldrich). Monoclonal antibodies were used at various dilutions (IgG1, 1:2,000; IgG2, 1:2,000; IgG3, 1:40,000; and IgG4, 1:60,000 in 0.5% casein sodium in phosphate-buffered saline containing 0.05% Tween 20), which gave similar optical density values for equal quantities of each IgG subclass. Peroxidase-conjugated streptavidin (1:1,000; Calbiochem, Billerica, MA) was added to the wells, and then incubated at 37°C for 1 hour.

Western blot, immunoprecipitation, and mass spectrometry. For each immunoprecipitation, five 100-mm poly-L-lysine–coated plates were seeded with primary oligodendrocyte precursor cells and were maintained for 10 days in vitro (the detailed protocol for oligodendrocyte differentiation is described in the e-Methods). Immunoprecipitation and mass spectrometry were then performed as previously described.[13]

Deglycosylation of NF155 was performed as previously described.[13] In some experiments, HEK cells were transfected with NF155 for 4 hours, then treated with tunicamycin (2 μg/mL) for 16 hours before solubilization. Fifty micrograms of proteins was loaded on 5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels, transferred, and immunoblotted with a mouse monoclonal anti-Myc antibody (1:1,000) or CIDP sera (1:500).

Sciatic and optic nerves from adult C57BL/6J mice were directly solubilized in SDS sample buffer, β-mercaptoethanol, and protease inhibitors. Samples were heated for 2 minutes at 92°C, then centrifuged for 10 minutes at 750*g*. Spinal cord extracts were prepared as previously described.[14] Protein concentration was determined using a BCA kit (Euromedex). Samples were loaded on a 5% SDS-PAGE gel, transferred, and immunoblotted with a rat polyclonal antibody against neurofascin recognizing both NF155 and NF186 (1:500)[15] or CIDP sera (1:500). Immunoreactivity was revealed using peroxidase-coupled secondary antibodies (1:5,000; Jackson ImmunoResearch) and BM chemiluminescence kit (Roche).

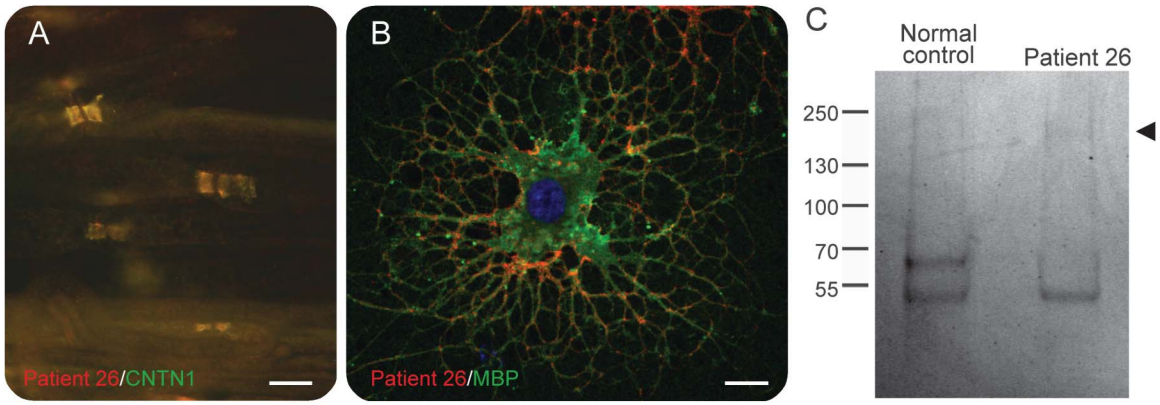
Immunohistochemical analyses. Detailed methods for immunohistochemical analyses are described in the e-Methods.

Statistics. Statistical analysis was performed using StatView version 5.0 (SAS Institute Inc., Cary, NC). A p value <0.05 was considered significant.

RESULTS

Identification of NF155 as a target for autoantibodies in CIDP. In a first screen, we identified a patient with CIDP who showed a strong IgG reaction against the paranodal regions of mouse sciatic nerve fibers (figure 1A). The IgG antibodies from this patient colocalized with CNTN1 at paranodes, but this patient did not show IgG antibodies against CNTN1 by ELISA. This suggested that the autoantibodies reacted against other paranodal components. To identify the target antigens, we tested the serum against cultures of neocortical neurons or mature myelinating oligodendrocytes, as these express many of the proteins found in peripheral axons and glial cells. The IgG antibodies did not bind neurons, but the IgG antibodies strongly reacted against surface antigens present on oligodendrocytes (figure 1B).

Figure 1 Glial NF155 is an autoantibody target in chronic inflammatory demyelinating polyneuropathy



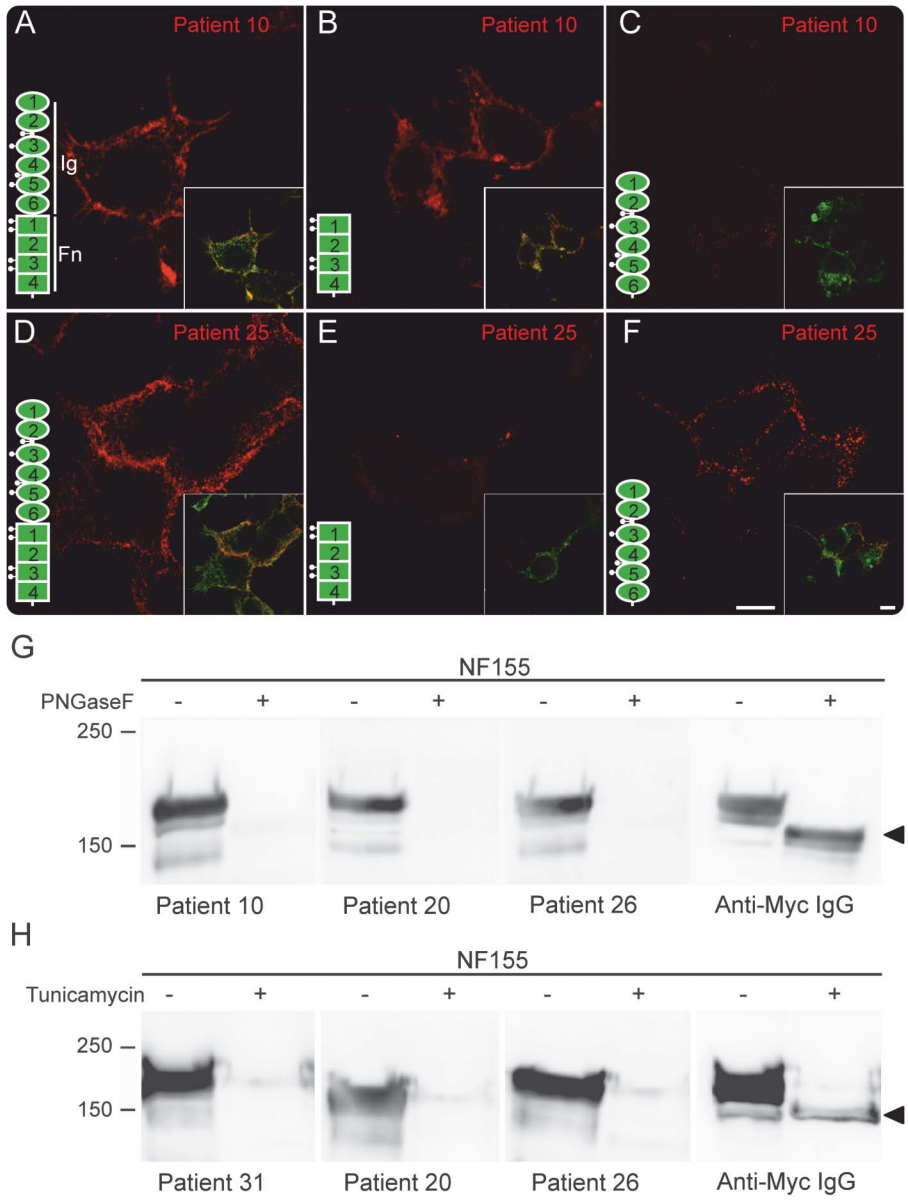
(A) These are mouse sciatic nerve fibers immunostained for IgG antibodies (red) from a patient with chronic inflammatory demyelinating polyneuropathy (patient 26) and for CNTN1 (green) to stain the paranodes. Human IgG antibodies bound the paranodal regions and colocalized with CNTN1. (B) The IgG antibodies (red) from this patient recognized a glial antigen at the surface of oligodendrocytes in culture labeled for MBP (green). Scale bar = 10 μm. (C) The target antigens were immunoprecipitated from cultured oligodendrocytes, separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, and stained with imperial blue. As control, IgG antibodies from a normal control (left) were used for immunoprecipitation. A protein band around 150–160 kDa (arrowhead) was specifically pulled down by the patient's IgG antibodies and identified as NF155 by mass spectrometry. Molecular weight markers are shown on the left in kilodalton. CNTN1 = contactin 1; IgG = immunoglobulin G; MBP = myelin basic protein; NF155 = neurofascin-155.

The target antigen was then immunoprecipitated from oligodendrocyte cultures with this serum. The CIDP IgG antibodies specifically recognized a faint protein band around 150 to 160 kDa (figure 1C), which was further identified as NF155 by mass spectrometry.

Association of CIDP with anti-NF155 IgG4 antibodies. These results prompted us to screen a large cohort of patients with CIDP (n = 533). IgG autoantibodies against NF155 were identified in sera from 48 patients with CIDP, but not in those from patients with GBS and those with MS as well as from healthy controls. IgG4 antibodies against NF155 were identified in 38 of the 48 sera (table e-1) and IgG3 antibodies against NF155 were detected in only one patient. In the 9 remaining patients, the IgG subclasses could not be determined. Four of the latter patients did not react against paranodes or NF155-transfected HEK cells, suggesting that these were false-positive. We thus focused our study on the 38 patients with anti-NF155 IgG4 antibodies. None of the 38 sera contained IgG antibodies against NF186 or CNTN1. Neither IgM nor IgA antibodies against NF155 were found in the 38 sera. Of note, the presence of anti-NF155 IgG4 antibodies was more frequent in CIDP than in GBS, MS, and normal control (7% [38/533] vs 0%; Fisher exact test, $p < 0.001$). In parallel, we blindly tested these sera on murine teased fibers and on NF155-transfected HEK cells. All the anti-NF155 IgG4-positive sera strongly reacted with the paranodes (figure e-1) and NF155-transfected HEK cells (figure 2, A and D). None of the sera with anti-NF155 IgG4 antibodies activated the complement pathway or induced the deposition of the immune complex on ELISA plates. These results suggest that anti-NF155 IgG antibodies do not fix complement.

Clinical features of anti-NF155 IgG4-positive CIDP. Table 1 shows the comparison of clinical features between anti-NF155 IgG4-positive, anti-CNTN1 IgG4-positive,[13] and CIDP patients without either antibody. The latter (n = 100) were randomly chosen. Anti-NF155–positive patients with CIDP were younger at onset than anti-CNTN1–positive patients or patients without either antibody (Mann–Whitney *U* test, $p < 0.001$), with 8 of the 38 (21%) showing an onset before 20 years of age (χ^2 test, $p < 0.01$). Initial rapid progression was more common in anti-NF155 IgG4-positive patients, although it did not reach statistical significance (χ^2 test, $p = 0.05$). The presence of anti-NF155 IgG4 antibodies was associated with sensory ataxia ($p < 0.001$; odds ratio [OR] = 14.7; 95% confidence interval [CI] = 5.9–36.1), tremor ($p < 0.001$; OR = 8.6; 95% CI = 3.1–23.7), and unresponsiveness to IVIg ($p < 0.005$; OR = 5.74; 95% CI = 1.9–17.5). In IVIg nonresponders, 4 (20%) had conduction block and 6 (30%) were corticosteroids responders, but did not respond to plasma exchange or other immunotherapy. Only 5 of 25 (20%) anti-NF155–positive patients responded to IVIg, whereas 33 of 56 (59%) antibodies-negative patients did. In all IVIg responders, corticosteroids (1 mg/kg/d or 1 g/d for 3 days) had been used at the same time. Thus, improvement may not solely be due to IVIg in these patients, and the response to IVIg may have been overestimated. In addition, 5 patients presented with cerebellar ataxia associated with nystagmus, which was higher than in

Figure 2 Anti-NF155 IgG antibodies target an *N*-glycosylated module



(A–F) Patients' IgG antibodies (red) were tested on living human embryonic kidney cells transfected with full-length Myc-tagged NF155 (A and D) or with constructs encoding solely the fibronectin type III (Fn) domains 1–4 (B and E) or the Ig domains 1–6 (C and F). A scheme of NF155 structure is inserted in each panel, showing the position of the putative *N*-glycosylation sites. Cells were then fixed and immunostained for Myc (green). Representative sera from patients with combined central and peripheral demyelination (patient 10) and chronic inflammatory demyelinating polyneuropathy (patient 25) are shown. The IgG antibodies from patient 10 (A–C) reacted against the Fn domains, whereas the IgG antibodies from patient 25 (D–F) recognize the Ig domains of NF155. The insets show the merge of the 2 channels (IgG and Myc). Scale bars = 10 μm. (G and H) Protein samples from NF155-transfected cells were untreated (−) or treated (+) with PNGaseF (G) or with tunicamycin (2 μg/mL; H). Proteins were then immunoblotted against Myc or representative sera from patients with chronic inflammatory demyelinating polyneuropathy (patients 10, 20, 26, and 31). The mouse anti-Myc antibodies recognized the *N*-glycosylated form of NF155 around 155 kDa and the unglycosylated protein core (arrowheads on the right). By contrast, patients' IgG antibodies only reacted against the *N*-glycosylated NF155. Molecular weight markers are shown on the left in kilodalton. IgG = immunoglobulin G; NF155 = neurofascin-155; PNGaseF = *N*-glycosidase F.

the antibodies-negative patients ($p = 0.001$; OR = 33.0; 95% CI = 1.7–612.7). Three of the 38 (8%) anti-NF155–positive patients had demyelinating lesions in the CNS (Fisher exact test, $p = 0.02$). As compared to the 13 anti-CNTN1 IgG4-positive patients with CIDP, modified Rankin Scale score was smaller (Mann–Whitney *U* test, $p < 0.001$). In general, the patients with CIDP who had anti-CNTN1 antibodies had more severe disease with an onset at older age as compared with anti-NF155–positive patients. However, they showed similar symptoms and responses to therapies (table e-2).

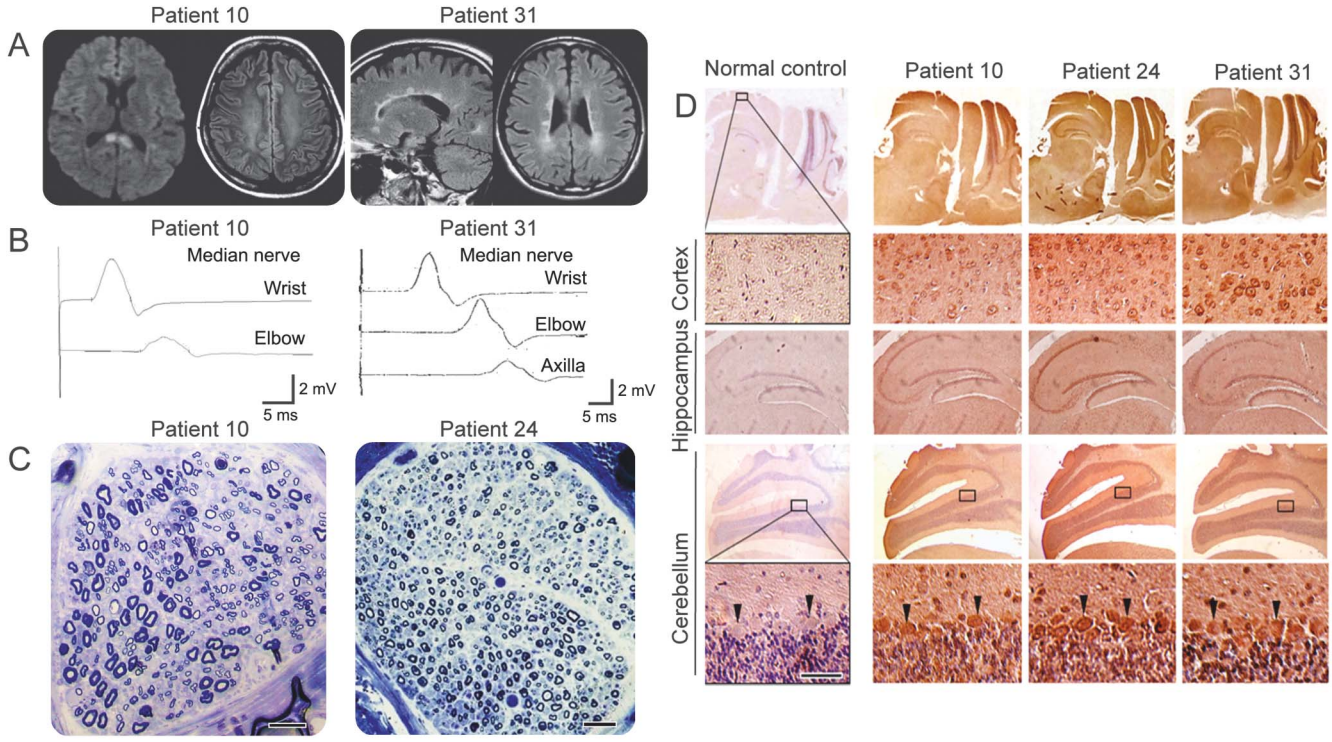
Table 1 Clinical features of CIDP associated with anti-NF155 and anti-CNTN1 IgG4 autoantibodies

	NF155 IgG4 (n = 38)	CNTN1 IgG4 (n = 13)	Ab negative (n = 100)	NF155 vs CNTN1, *p* value	NF155 vs Ab negative, *p* value
Age at onset, y	31 (10-67)	60 (33-81)	48 (6-83)	<0.001	<0.001
Younger than 20 y, n (%)	8 (21)	0 (0)	5 (5)	0.09	<0.01
Sex, male, n (%)	27 (71)	10 (77)	58 (58)	0.96	0.23
Subacute onset, n (%)	12 (32)	3 (23)	15 (15)	0.86	0.051
Sensory ataxia, n (%)	28 (74)	13 (100)	16 (16)	0.10	<0.001
Tremor, n (%)	16 (42)	2 (15)	7 (7)	0.16	<0.001
Cerebellar ataxia, n (%)	5 (13)	1 (8)	0 (0)	0.98	<0.005
CNS demyelination, n (%)	3 (8)	0 (0)	0 (0)	0.56	0.02
Modified Rankin scale	2.0 (1-5)	4.0 (3-5)	2.0 (1-6)	<0.001	0.24
Good response, n (%)					
IVIg	5/25 (20)	4/10 (40)	33/56 (59)	0.43	<0.005
Corticosteroids	15/29 (52)	8/11 (73)	19/42 (45)	0.40	0.78

Abbreviations: Ab = antibody; CIDP = chronic inflammatory demyelinating polyneuropathy; CNTN1 = contactin 1; IgG4 = immunoglobulin G4; IVIg = IV immunoglobulin; NF155 = neurofascin-155.

In summary, anti-NF155 IgG4 antibodies were associated with a subgroup of CIDP patients showing a younger age at onset, sensory ataxia, tremor, and a poor response to IVIg treatment. In some patients, we also found that anti-NF155 IgG4 was associated with CNS demyelination (figure 3A). Case reports of the 3 patients who presented with concomitant CNS demyelination can be found in appendix e-1.

Figure 3 Laboratory findings of patients with combined central and peripheral demyelination



(A) Diffusion-weighted images in patient 10 showed signal abnormalities in the splenium of the corpus callosum. Fluid-attenuated inversion recovery images in patients 10 and 31 showed multiple sclerosis–like lesions in the juxtaventricular regions. (B) Median nerve motor conduction studies showed prolonged distal latencies and reduced conduction velocities with reduced amplitude and probable conduction block. (C) These are transverse sections of sural nerve biopsies stained with toluidine blue. Sural nerve biopsies revealed a moderate decrease in the number of large- and small-diameter fibers, some demyelinating changes, and axonal degeneration, but no cellular infiltration or onion-bulb formation. Scale bars = 50 μm. (D) The immunoreactivity of serum immunoglobulin G antibodies was examined in mouse cortex, hippocampus, and cerebellum, counterstained with hematoxylin. Note that the 3 sera strongly labeled Purkinje cells (arrowheads). Scale bar = 50 μm.

Binding of anti-NF155 antibodies to CNS tissues and myelinated tracts. Because patients 10, 24, and 31 had central and peripheral demyelination (figure 3, A–C), binding of their sera to murine brain was investigated. IgG antibodies from these patients showed intense reactivity against cortex, hippocampus, and cerebellum (figure 3D). In the cerebellum, the reactivity predominated in the Purkinje cells and granular layers for all the patients. IgG antibodies from patients with CIDP, who carried anti-NF155 IgG4 antibodies but no central demyelination, had similar reactivity (data not shown). Furthermore, we found that patients with CIDP or CCPD react similarly against NF155 from peripheral or central paranodes (appendix e-1).

Binding of CIDP and CCPD autoantibodies to *N*-glycosylated modules of NF155. We then examined whether the IgG antibodies from the patients target different epitopes within NF155. NF155 comprises 4 fibronectin type III (Fn) domains (Fn1–4) and 6 Ig domains (Ig1–6). To target NF155 epitopes, we first deleted the Fn1–4 domains and the Ig1–6 domains. Thirteen (patients 26–38) of the 38 sera reacted against neither the Fn1–4 nor the Ig1–6 domains alone, but recognized solely the full-length protein (table e-1). This suggested that these IgG antibodies react against conformational epitopes requiring both the Fn and Ig domains. Then, we found that 17 (patients 1–17) of the 38 sera reacted selectively against the Fn1–4 domains, 5 (patients 18–22) recognized both Ig1–6 and Fn1–4 domains, and 3 (patients 23–25) reacted selectively against the Ig1–6 domains. Representative results are shown in figure 2, A–F. Of the 38 patients, the serum IgG from 30 patients (79%) did not bind NF155 deleted from its Fn1–4 domain. This indicated that the Fn1–4 domain contains the main target epitope. No clear correlations could be drawn between antibody reactivity and the clinical phenotype of the patients.

We further investigated whether the glycosylation of NF155 might influence IgG binding. For that purpose, we performed deglycosylation experiments using peptide *N*-glycosidase F, and in some experiments, we blocked *N*-glycosylation using tunicamycin. In transfected HEK cells, NF155 appeared mostly as a single protein band around 155 kDa (figure 2, G and H), which migrated at a lower apparent molecular weight after treatment with peptide *N*-glycosidase F or tunicamycin. By contrast, CIDP and CCPD sera recognized solely the glycosylated NF155 and did not react against the unglycosylated or deglycosylated proteins. This further confirmed that sera from most patients reacted against a common epitope, which is *N*-glycosylated.

DISCUSSION In the current study, we screened a large cohort of patients with CIDP for anti-NF155 reactivity. Anti-NF155 IgG4 antibodies were significantly associated with 7% of CIDP patients. This frequency was slightly more important than that found in 2 smaller cohorts (3% [4/119 cases] and 4% [2/53])[2,3] and was more important than the prevalence of anti-CNTN1 antibodies (2.4%) in our own cohort.[13] Our findings corroborate many of the clinical features previously described, namely, most patients were unresponsive to IVIg and many presented with disabling characteristic tremor (high amplitude, low frequency, postural, and intention).[3] However, the clinical features of the patients with CIDP who had anti-NF155 IgG4 antibodies appear more complex in our study than those originally reported. First, patients with anti-NF155 IgG4 antibodies were significantly younger than those with anti-CNTN1 IgG4 antibodies and those without either antibody. Twenty-one percent of anti-NF155 IgG4-positive patients in our cohort were younger than 20 years but none in the 2 previous studies.[2,3] Second, anti-NF155 IgG4 antibodies were statistically associated with sensory ataxia, as were anti-CNTN1 IgG4 antibodies,[13] but also with tremor. Some patients presented with positional tremor, whereas others had intentional tremor. In addition, 5 patients had cerebellar ataxia, dysarthria, and nystagmus. These symptoms, as well as CNS demyelination as discussed below, are highly unusual for CIDP, but they are not exclusion criteria in CIDP diagnosis.[9] Moreover, each patient met the electrodiagnostic criteria for CIDP, as shown for patients 10 and 31 (figure 3 and appendix e-1).

In the current study, we detected CNS demyelination in 8% of patients with CIDP and anti-NF155 IgG4 antibodies, but not in patients with anti-CNTN1 IgG4 antibodies or in patients without either antibody. This suggests that the reactivity against NF155 helps in prognosticating CNS disorders in patients with CIDP. Brain and spinal cord lesions are not commonly encountered in typical CIDP. However, several studies reported cases of patients with demyelinating lesions appearing concurrently or sequentially in the CNS and peripheral nervous system (PNS).[16] Recent reports showed that 46% (5/11) and 71% (5/7) of Japanese patients with CCPD carried anti-NF155 IgG antibodies.[7,8] The age at onset was between 10 and 48 years in their cohorts and was in keeping with our observations (21–33 years for ours). However, the patients with CCPD in our cohort did not always respond to IVIg, whereas those in their cohort did. One possible explanation is that the IgG subclasses were different in these 2 cohorts. Our patients showed IgG4 antibodies, which have a low affinity for Fc receptors and complement.[17] By contrast, the IgG subclass has not been examined in the other 2 studies. All the patients in our cohort required maintenance of

prednisolone and additional immunosuppressant for disease control, despite repeated IVIg infusions. Relapse occurred in one patient when prednisolone was tapered.

We earlier discussed the possibility of an antigen blocking effect of anti-CNTN1 antibodies, which may preferentially affect the sensory axon paranodes. A recent report indicates that patients with anti-CNTN1 IgG4 antibodies showed specific paranodal alterations in dermal nerve biopsies.[18] Furthermore, anti-CNTN1 antibodies altered paranodal junctions in myelinating cocultures of Schwann cells and dorsal root ganglions.[19] As a partner of CNTN1 at the paranodes, anti-NF155 antibodies may demonstrate similar antigen blocking effect and inhibit the interaction between NF155 and CNTN1/Caspr1 or other partners. However, it remains unclear why IgG4 preferentially affects PNS axons in some patients or affects both PNS and CNS axons in others.

We found that the Fn1–4 domain of NF155 is the main target epitope in 79% of the patients. In a previous report, anti-NF155 IgG4 antibodies also targeted the Fn3–4 domains in 2 patients.[2] We further found that the anti-NF155 IgG antibodies recognized an *N*-glycosylated epitope. This further indicates that anti-NF155 IgG4-positive patients appear to form a homogeneous subgroup with a common epitope. Because all the patients presented with CIDP, the Fn domains of NF155 likely have a crucial function in peripheral nerve physiology. The function of the Fn domains of NF155 is unclear. The interaction between CNTN1 and NF155 appears to be mediated through their respective Ig domains,[20] which are the target of anti-CNTN1 IgG4 antibodies.[13] Nonetheless, NF155 has been found to favor the cell adhesion of Schwann cells or dorsal root ganglion neurons, notably through the Fn3 domain.[21] Thus, this domain may bind glial/axonal partners and have a role in paranode formation or its stability.

Only 20% of anti-NF155 IgG4-positive patients responded to IVIg. This was much below the percentage of responsive patients in our negative group (59%) or in reported studies (54%–63%).[1,22–24] Similar observations were reported by a Spanish group, in which none of the 4 patients responded to IVIg.[3] In contrast, beneficial response was noted after plasma exchange in another study.[2] As mentioned above, IgG4 antibodies do not bind C1q and have low affinity to Fc receptors.[17] One of the major mechanisms of IVIg is the inhibition of complement pathway,[25,26] so the poor response in anti-NF155 IgG4-positive patients to IVIg is not unexpected. International multicenter studies should be performed in the future to investigate this subgroup of patients with CIDP and to define first-line therapies.

AUTHOR CONTRIBUTIONS

Drafting the manuscript for content, including medical writing for content: J.J.D., Y.M., Y.F., T.I., K.S., N.K., H.I., A.H.Y.W., and N.Y. Revising the manuscript for content, including medical writing for content: J.J.D., A.H.Y.W., and N.Y. Study concept and design: J.J.D. and N.Y. Acquisition of data: J.J.D., Y.M., Y.F., C.M., T.I., M.B., H.I., K.S., and N.K. Analysis and interpretation of data: J.J.D., Y.M., Y.F., and N.Y.

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DISCLOSURE

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