Neurofascin-155 Immunoglobulin Subtypes
Clinicopathologic Associations and Neurologic Outcomes

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Abstract

Background and Objective
Multiple studies highlighting the diagnostic utility of neurofascin-155 (NF155)–immunoglobulin G4 (IgG4) in chronic demyelinating inflammatory polyradiculoneuropathy (CIDP) have been published. However, few studies comprehensively address the long-term outcomes or clinical utility of NF155–immunoglobulin M (IgM) or NF155–immunoglobulin G (IgG) in the absence of NF155-IgG4. We evaluated phenotypic and histopathologic specificity and differences in outcomes between these NF155 antibody isotypes or IgG subclasses. We also compare NF155-IgG4-seropositive cases to other seropositive demyelinating neuropathies.

Methods
Neuropathy patient sera at Mayo Clinic were tested for NF155-IgG4, NF155-IgG, and NF155-IgM autoantibodies. Demographic and clinical data of all seropositive cases were reviewed.

Results
We identified 32 NF155 cases (25 NF155-IgG-positive [20 NF155-IgG4-positive], 7 NF155-IgM-seropositive). NF155-IgG4-seropositive patients clinically presented with distal more than proximal muscle weakness, positive sensory symptoms (prickling, asymmetric paresthesia, neuropathic pain), and gait ataxia. Cranial nerve involvement (11/20 [55%]) and papilledema (4/12 [33%]) occurred in many. Electrodiagnostic testing (EDX) demonstrated demyelinating polyradiculoneuropathy (19/20 [95%]). Autonomic involvement occurred in 45% (n = 9, median composite autonomic scoring scale score 3.5, range 1–7). Nerve biopsies from the NF155-IgG4 patients (n = 11) demonstrated grouped segmental demyelination (50%), myelin reduplication (45%), and paranodal swellings (50%). Most patients needed second- and third-line immunosuppression but had favorable long-term outcomes (n = 18). Among 14 patients with serial EDX over 2 years, all except one demonstrated improvement after treatment. NF155-IgG-positive, NF155-IgG4-negative (NF155-IgG-positive) and NF155-IgM-positive patients were phenotypically different from NF155-IgG4-seropositive patients. Sensory ataxia, neuropathic pain, cerebellar dysfunction, and root/plexus MRI abnormalities were significantly more common in NF155-IgG4-positive compared to myelin-associated glycoprotein (MAG)–IgM neuropathy. Chronic immune sensory polyradiculopathy (CISP)/CISP-plus phenotype was more common among contactin-1 neuropathies compared to NF155-IgG4-positive cases. NF155-IgG4-positive cases responded favorably to immunotherapy compared to MAG-IgM-seropositive cases with distal acquired demyelinating symmetric neuropathy (p < 0.001) and had better long-term clinical outcomes compared to contactin-1 IgG (p = 0.04).
Neurofascin-155 (NF155) autoantibodies are among the most common nodal and paranodal antibodies, comprising 4% to 18% of all chronic demyelinating polyradiculoneuropathy (CIDP) cases. Despite the growing utilization of these antibodies in clinical practice, studies evaluating long-term outcomes and histopathologic characterization are limited.

In this study, we determine frequency of NF155 autoantibodies in a large demyelinating neuropathy cohort. We evaluate phenotypic and histopathologic specificity and differences in outcomes between NF155–immunoglobulin G4 (IgG4)–seropositive, NF155–pan–immunoglobulin G (IgG), and NF155–immunoglobulin M (IgM)–seropositive cases. We also compare phenotypic differences and outcomes in NF155–IgG4–positive cases to myelin-associated glycoprotein (MAG)–IgM and contactin-1–IgG–associated demyelinating neuropathies.

Methods
Our primary research question was to evaluate the clinical utility of NF155–IgG4 and NF155–IgM or NF155–IgG (in the absence of NF155–IgG4) autoantibodies and assess phenotypic and histopathologic differences in long-term outcomes among patients with these autoantibodies.

Patient Selection
We retrospectively reviewed the Mayo Clinic Rochester database for diagnostic codes designating demyelinating neuropathies from January 1, 1986, to January 1, 2019. On review of electronic medical records of the screened cases, 237 acute or chronic inflammatory demyelinating polyradiculoneuropathies (AIDP [n = 23], CIDP [n = 214]) were identified (cohort 1). An additional 173 patients with stored sera identified during this initial screening with an alternative neuropathy etiology or clinical presentation not consistent with AIDP/CIDP/chronic immune sensory polyradiculopathy (CISP)/CISP-plus phenotype were used as disease controls (eFigure 1, http://links.lww.com/WNL/B609). Peripheral nerve specialists (S.S., D.D., C.K., M.L.M., S.E.B., P.J.B.D.) prospectively sent samples for NF155 autoantibody evaluation among cases where nodal and paranodal antibody-mediated neuropathy was suspected between January 1, 2019, and March 31, 2021 (cohort 2).

NF155 Testing
Cohort 1
Sera of all patients were tested on in-house flow cytometry–based assay that utilized a stable cell line coexpressing human NF155 and GFP using a previously described approach. Patient and control sera were tested at 1:10 and 1:40 dilutions for NF155–IgG4 and NF155–IgG, respectively. The median fluorescence intensity of Alexa Fluor 647–conjugated anti-human IgG4 or IgG was determined for both nontransfected and transfected cells. The ratio of median fluorescence intensity values for green fluorescent protein (GFP)+ and GFP− cells was calculated as IgG or IgG4 binding index. IgG binding index of ≥5 and IgG4 binding index ≥2 were considered positive.

Cohort 2
Prospectively collected patient sera were evaluated by flow cytometry for NF155–IgG4 in-house or for NF155 IgG4, pan-IgG, and IgM antibodies at the Washington University clinical service neuromuscular laboratory by Western blot analysis.

Nerve Histology
Nerve biopsies from NF155 autoantibody–positive cases were performed and processed in our peripheral nerve laboratory using standard histologic stains and immunohistochemistry stains (CD45, CD68) prepared on paraffin and epoxy sections. Teased fibers were prepared and graded
by previously defined pathologic criteria. Morphometric analysis was performed on epoxy sections using our Imaging System for Nerve Morphometry.

**Clinical Outcome**

Inflammatory Neuropathy Cause and Treatment (INCAT) disability scores and gait assistance (assessed at final neurologic visit) were calculated for all patients. Favorable outcomes were defined by improvement ≥1 in INCAT disability scores calculated before and after immunotherapy. We defined disease relapse as >3 units increase in INCAT disability score after at least 3 months of disease stability when follow-up data were available.

**Clinical Comparison Group**

NF155-IgG4-seropositive (NF155-IgG4-positive) cases were compared to contactin-1-IgG and MAG-IgM-seropositive patients evaluated at Mayo Clinic within the study period. Contactin-1-IgG cases evaluated at Mayo Clinic between January 1, 1993, and March 31, 2021 (4 Mayo patients previously published) were included for clinical comparisons. Contactin-1 Euroimmun CBA was utilized for contactin-1 IgG and IgG4 testing. We searched the Mayo Clinic electronic database for patients seen in the neuromuscular and peripheral nerve specialty clinics between 2004 and 2018 with a distal acquired demyelinating symmetric neuropathy phenotype in the setting of an IgM monoclonal gammopathy of uncertain significance (n = 86). MAG IgM antibodies were measured using a clinically validated ELISA from Bühlmann Diagnostics. Positivity was defined using a cutoff of 1,500 Bühlmann titer units.

**Statistical Analysis**

For comparisons between groups, qualitative and continuous variables were analyzed using the Fisher exact test and Mann-Whitney U test, respectively.

**Standard Protocol Approvals, Registration, and Patient Consents**

The study was approved by the institutional Review Board of the Mayo Clinic, Rochester, Minnesota (institutional review board number 08-006647). Permission to participate was obtained from the patients.

**Data Availability**

Anonymized data not published within this article will be made available by request from any qualified investigator.

**Results**

**Retrospective and Prospective Cohorts: Classification of NF155 Isotypes**

**Cohort 1**

Among 214 patients with CIDP and 23 patients with AIDP evaluated from the retrospective cohort, 14 patients with CIDP were NF155-IgG4-positive (6.5%); none of the patients with AIDP tested positive. Three patients were NF155-IgG-positive and NF155-IgG4-negative (NF155-IgG-positive). All 173 disease control sera tested for NF155-IgG4 by flow cytometry were negative; 1 patient was NF155-IgG-positive.

**Cohort 2**

Fourteen NF155 autoantibody–positive patients were evaluated prospectively by peripheral nerve specialists. Six patients were NF155-IgG4-positive. One patient was only NF155-IgG-positive. An additional 7 patients were NF155-IgM-positive on Western blot but NF155-IgG- and NF155-IgG4-negative.

**Neuropathic Pain, Distal Weakness, Sensory Ataxia, Tremor, and Cranial Nerve Involvement Were Common Among NF155-IgG4-Positive Patients**

Demographic and clinical features for all NF155-IgG4-positive patients are shown in Table 1. Median age at symptom onset was 45.5 years, with 1 patient having symptom onset at 4 years of age. Nadir of symptoms severity occurred up to 12 months from symptom onset. Median time from symptom onset to demyelinating neuropathy diagnosis was 10 months (range 1–208 months). As a group, the most common clinical features were distal more than proximal muscle weakness (14 [70%]). A majority of the patients had distal more than proximal weakness (14 [70%]). Motor deficits involving lower extremities (18 [90%]) were much more common than upper extremity predominant presentations. Positive sensory disturbances (including pricking, asymmetric paresthesia, and neuropathic pain) with sensory as well as cerebellar ataxia and tremor were observed (10 [50%]). The tremor was an action tremor affecting the distal extremities, most often the fingers and hands. Cranial nerve and bulbar symptoms were common in 55% (11/20) and included one or more of the following: dysphagia or dysarthria (n = 7), diplopia or ptosis (n = 2), blurry vision (n = 1), and unilateral facial numbness (n = 2). Papilledema was also present (4/12 [33%]). Neurologic examination findings are shown in Table 1.

**Evaluation of NF155-IgG4-Seropositive Patients Revealed Demyelinating Polyradiculoneuropathy, Frequently With Autonomic Dysfunction**

Nerve conduction studies (NCS) and needle EMG examinations were performed in all patients. All NF155-IgG4-seropositive cases had electrodiagnostic features consistent with demyelinating polyradiculopathy with slowed motor conduction velocities or prolonged distal latencies. All 20 patients met the European Federation of Neurologic Societies/Peripheral Nerve Society demyelinating electrophysiologic criteria for CIDP (18 definite, 1 probable, and 1 possible). Polyradiculoneuropathy with evidence of root and peripheral nerve involvement was the most common electrodiagnostic phenotype (n = 19 [95%]). More specifically, all patients had reduced or absent motor amplitudes (n = 20 [100%]), reduced or absent sensory amplitudes (n = 19 [95%]), and often prominent slowing of motor conduction velocities (n = 18 [90%]). F-wave
Latencies were prolonged (n = 16) or absent (n = 2) in 90% of patients (n = 18 [90%]). Other features noted in a minority of cases were temporal dispersion (n = 4 [20%]) and conduction block (n = 4 [20%]). Blink reflex studies were performed in 19 cases and were prolonged in a majority of patients (84% [16/19]). Median R1 latency was 17.3 milliseconds (range 11.6–27.4 milliseconds). In nearly all cases, fibrillation potentials were identified in proximal/paraspinal muscles, supporting radicular localization (n = 19 [95%]).

Autonomic symptoms were present in 45% (9/20) of patients and included orthostatic intolerance (defined by clinical history of syncope or lightheadedness) and blood pressure changes (n = 3), urinary incontinence and constipation (n = 3), and erectile dysfunction temporarily associated with the date of symptom onset (n = 2). Six patients demonstrated abnormal autonomic reflex screen testing with median composite autonomic scoring scale (CASS) of 3.5 (range 1–7), indicating a moderate autonomic neuropathy. Testing demonstrated sudomotor dysfunction (n = 5) as well as cardiovascular adrenergic impairment (n = 3).

CSF studies were available in 19 NF155-IgG4-positive patients. CSF protein was elevated in all patients with median CSF protein of 224 g/dL (range 60–834 g/dL, normal <50 mg/dL). Nine patients had mild lymphocytic pleocytosis (median 5 cell/mm³; range 4–100 cells/mm³).

### MRI Showed Diffuse Symmetric Enlargement, Nerve Root Thickening, and Nerve Root/Plexus Gadolinium Enhancement Among NF155-IgG4-Seropositive Patients

MRIs were available to review in 17 NF155-IgG4 patients (spine, n = 13 and plexus, n = 9). Diffuse symmetric enlargement of the lumbosacral plexus or lumbosacral roots with marked T2 hyperintensity were a common finding in 82% (14/17) of patients. The most common abnormality identified on lumbar spine MRI was nerve root thickening (85% [11/13]) followed by nerve root gadolinium enhancement on axial images (46% [6/13]). In one patient, patchy, nonenhancing T2 hyperintensity was seen throughout the cervical spinal cord up to the brainstem. One patient had abnormal signal involving the oculomotor, trigeminal, facial, and vestibulocochlear nerves bilaterally on head MRI. Six patients had abnormal white matter changes that were considered as nonspecific, presumed to be secondary to small vessel disease. Overall, there were no radiologic findings supporting a concurrent central demyelinating disease in the 9 patients who underwent head MRI.

### Histopathologic Analysis of NF155-IgG4-Seropositive Patients Showed Increased Rate of Segemental Demyelination, Active Axonal Degeneration, and Paranodal Expansions

Nerve biopsies of 11 NF155-IgG4-positive patients were performed at our institution. Median time from symptom to biopsy was 9 months (range <1–43 months). The biopsies included lumbar dorsal rootlet (n = 1), fascicular sciatic (n = 1), and distal cutaneous nerve biopsies (n = 11). Electron microscopy was performed to better demonstrate ultrastructural changes in one patient who had proximal and distal biopsy.

Teased fiber preparations were available for review in 10 of 11 NF155-IgG4-positive biopsies and showed increased rates of segmental demyelination (n = 5), highest in the fascicular sciatic biopsy (48%; Figure 1D) and lowest in distal cutaneous biopsies (mean 6%; range 0–25%, normal <2%). Increased frequencies of myelin reduplication were common and observed in 50% (n = 5) of biopsies, with areas of paranodal swellings (areas of thickened myelin adjacent to the node of Ranvier) in 50% (n = 5) (Figure 1, A–C), ranging in severity from most severe in the proximal site (Figure 1C) to less severe areas distally (Figure 1, A and B).

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**Table 1** Demographic Clinical Characteristics of NF155-IgG4-Seropositive Patients

| Category                                | NF155-IgG4 (n = 20) |
|-----------------------------------------|---------------------|
| Male                                    | 11 (55)             |
| Age at symptoms onset, y                | 45.5 (4–72)         |
| Follow-up, mo, mean (range)             | 85 (5–411)          |
| Time to diagnosis, mo                   | 10.5 (<1–208)       |
| Sensory pricking or paresthesia         | 17 (85)             |
| Neuropathic pain at onset               | 9 (45)              |
| Cerebellar features                     | 4 (20)              |
| Autonomic symptoms                      | 9 (45)              |
| Sensory ataxia on examination           | 18 (90)             |
| CN involvement on examination           | 11 (55)             |
| Tremor or pseudoathetosis               | 10 (50)             |
| Large fiber sensory loss on examination | 18 (90)             |
| Small fiber sensory loss on examination | 18 (90)             |
| Muscle weakness on examination          | 19 (95)             |
| Loss of deep tendon reflexes           | 18 (90)             |
| Papilledemaa                            | 4 (33)              |
| CSF protein, mg/dL                      | 224 (60–834)        |
| Demyelinating features by EFNS<sup>a</sup> | 20 (100)          |
| Nerve root/plexus enhancement on MRI<sup>c</sup> | 14 (82)         |
| Gait aid dependent at first follow-up   | 15 (75)             |
| Gait aid dependent at last follow-up    | 8 (40)              |

*Abbreviations: CN = cranial nerves; EFNS = European Federation of Neurological Societies; IgG4 = immunoglobulin G4; NF155 = neurofascin-155. Values are n (%) or median (range) unless otherwise specified.
*<sup>a</sup>Twelve patients had funduscopic examination.
*<sup>b</sup>Initial EMG/nerve conduction study data utilized.<sup>18</sup>
*<sup>c</sup>Seventeen patients had MRI available to review.
On the paraffin-embedded sections, the main pathologic abnormality was loss of large, myelinated nerve fibers seen in both distal and proximal biopsies (Figure 1A). Onion-bulb formations were not observed in any of the distal biopsies. Multifocal fiber loss was seen in 4/11 biopsies and subperineurial edema (Figure 2B) was present in 2 biopsies. Individual to small endoneurial and epineurial inflammatory collections were seen in 81% (9/11) of the distal nerve biopsies. In one patient (with severe sensory ataxia, tremor, and pseudoathetosis) who had 2 biopsies, a proximal fascicular sciatic biopsy and a sural nerve biopsy, large collections of epineurial mononuclear cells were seen in the proximal fascicular sciatic biopsy (Figure 2, C and D), with less inflammatory infiltrate in the distal sural site. Fascicular sciatic biopsy from another patient demonstrated multiple small endoneurial and epineurial inflammatory cell collections. The overall density of myelinated fibers ranged from normal to severely reduced.

**NF155-IgG4-Seropositive Cases Were IVIg Refractory, With Favorable Response to Second-Line Immunosuppressive Agents**

All NF155-IgG4-positive patients were treated with immunotherapy. First-line immunotherapies included one or more of the following: IV immunoglobulin (IVIg, n = 18) alone or in combination with IV methylprednisolone (IVMP, n = 7) and plasmapheresis (PLEX, n = 2). Most cases (12/18) had initially favorable responses to IVIg but later became IVIg refractory despite continued treatments. Six patients had worsening of their symptoms even with initial IVIg treatments.

A subset of these patients required secondary long-term immunosuppression (n = 18 [90%]): mycophenolate mofetil (n = 7), rituximab (n = 5), azathioprine (n = 4), or cyclosporine (n = 2). Eight cases needed a third immunotherapy—corticosteroids (n = 4), plasmapheresis (n = 2), or IVlg (n = 2)—while on immunosuppression medications. As a group, most patients improved at the time of last follow-up (n = 18).

**Relapses Were Common in NF155-IgG4-Seropositive Patients and Half of the Patients Required Gait Aid by 1 year, Indicating High Morbidity**

Median duration of follow-up among NF155-IgG4-positive cases was 7 years (range 5–411 months) measured from symptom onset to last follow-up or death. Most of these cases had >3 years of follow-up with repeated examinations (n = 18 [90%]). Despite refractoriness to IVIg, long-term outcomes in most cases were favorable (n = 18 [90%]). One patient stabilized and one continued to deteriorate despite IVMP and azathioprine treatment. Death was reported in 3 patients (12%) by the end of the study period. One (patient with fascicular sciatic biopsy) died from disease complication due to central port infection after plasmapheresis treatment, 1 from septic shock due to diverticulitis, and cause of death was unclear in 1 patient who was lost to neurology follow-up.

Median number of relapses per patient was 1 (range 1 to 3 times). Median time to relapse was 58 months (range 11–220 months). Nearly 50% of patients experienced relapses in the first 4 years from symptom onset and in the first 2 years from starting...
treatment (Figure 3A). There was a significant difference in Inflammatory Neuropathy Cause and Treatment (INCAT) disability scores comparing patients who had relapse vs patients who did not experience relapses in their disease course ($p = 0.02$; Figure 3B).

Most patients were using gait aids at neurologic presentation ($n = 15$ [75%]): cane ($n = 1$), walker ($n = 5$), or wheelchair ($n = 9$). At the time of last follow-up, 7 patients who previously used gait aids were independently ambulatory. Of the remaining 8 patients, 1 was using a cane, 3 wheelchairs, and 4 walkers at the time of last follow-up. Neurologic clinical outcomes measured at last neurology visit including needing assistance in daily tasks was markedly better after treatment ($p < 0.001$, confidence interval 1–5). Time from symptom onset to maximal gait aid dependence was reached in the first year of the disease in half of patients, signifying disease nadir (Figure 4A). Electrophysiologic testing with serial nerve conduction evaluations was available in 14 patients and the summed compound motor action potential (CMAP), a calculation in which CMAP amplitudes of all recurrent motor nerve fibers are summed (a rough measure of axonal integrity), showed improvement after $>24$ months of treatment in NF155-IgG4-positive cases (Figure 4B).

**Clinical Comparison of Nodoparaneopathies to Other Seropositive Demyelinating Polyradiculoneuropathies**

To further characterize the clinical phenotype and to identify distinguishing features, we compared NF155-IgG4-positive patients to those with contactin-1–IgG-positive and MAG-IgM-seropositive demyelinating polyneuropathy (Table 2). NF155-IgG4-positive onset age was significantly younger compared to MAG-IgM ($p < 0.001$). Sensory ataxia, neuropathic pain, cerebellar dysfunction, and root/plexus MRI abnormalities were significantly more common in NF155-IgG4-positive compared to MAG-IgM neuropathy. CISP/CISP-plus phenotype was more common among contactin-1 neuropathies compared to NF155-IgG4-positive cases. INCAT disability scores at first encounter were significantly worse in the NF155-IgG4-positive compared to MAG-IgM groups ($p < 0.001$). Improvement in INCAT disability score between first and last INCAT was significantly greater in NF155-IgG4-positive patients than MAG-IgM neuropathy. CISP/CISP-plus phenotype was significantly more common in NF155-IgG4-positive cases (Figure 4B).

**NF155-IgG4-Negative Patient Characteristics**

**NF155-IgG-Positive or IgM-Positive Patients Were Not Clinically Distinct**

A total of 5 patients were NF155-IgG-positive (NF155-IgG4-negative): 4 in retrospective cohort (1 disease control) and 1 in prospective cohort. The median age at diagnosis was 56.5 years (range 30–66 years). Median time from symptom onset to diagnosis was 11 months (range 4–61 months). Clinical presentations
were consistent with motor predominant polyradiculoneuropathy (n = 2), subacute motor polyradiculoneuropathy (n = 1), length-dependent sensorimotor peripheral neuropathy (n = 1), and length-dependent sensory neuropathy (n = 1).

We identified 7 cases prospectively who were negative for NF55-IgG but NF55-IgM-positive. The median age at presentation of this group was 54 years (range 21–63 years). Clinically, they had various neurologic presentations, including motor-predominant axonal polyradiculoneuropathy (n = 2), sensory motor predominant axonal polyradiculoneuropathy (n = 1), sensorimotor length-dependent axonal polyneuropathies (n = 1), length-dependent sensory predominant axonal polyneuropathy (n = 1), brachial plexopathy (n = 1), and facial numbness (n = 1).

NF155-IgG-Positive or IgM-Positive Cases had No Distinctive Electrophysiologic, CSF, or Imaging Findings

Electrodiagnostic findings included prolonged F-wave latency (n = 1), reduced distal sensory and motor amplitudes (n = 2), and reduced motor amplitudes (n = 2). Conduction velocity showed slowing (n = 2) or was normal (n = 3). Blink studies were abnormal in 2 of 3 patients tested.

**Figure 3** Relapses Versus Time From Diagnosis in NF155-IgG4-Positive Patients

Survival analysis comparison showing time to relapse from symptoms onset compared between 2 para/nodal antigens contactin-1 (CNTN1) and neurofascin-155 (NF155)-immunoglobulin G4 (IgG4)-positive patients. We demonstrate 50% of relapses occur in the first year from symptoms onset. The broken line stands for the median for each group (A). Whisker box plot comparison of Inflammatory Neuropathy Cause and Treatment (INCAT) scores at first neuromuscular visit shows higher score (more deficits) in those with relapses (p = 0.02) (B).

**Figure 4** Electrophysiologic and Clinical Outcomes NF155-IgG4 and Contactin-1–IgG Cohorts

Time to maximal gait aid needed from symptoms onset comparing neurofascin-155 (NF155) and contactin-1 (CNTN1) positive patients (A), demonstrating that in the first 6 months, 50% of patients will reach disease nadir. (B) Compound motor action potential (CMAP) comparison with 4 intervals of time showing return to baseline more than 2 years after symptoms onset evaluated at our EMG laboratory.
Among the NF155-IgM-positive patients, electrodiagnostic findings were length-dependent axonal polyneuropathy (n = 3), mixed axonal and demyelinating polyneuropathy (n = 2), upper trunk brachial plexopathy (n = 1), and normal (n = 1). Blink studies were normal in all tested patients (n = 5).

**CSF Studies**

CSF studies in NF155-IgG-positive patients were performed in 4 patients. CSF protein levels were elevated in 2 of the 4 patients; median CSF protein level was 200 g/dL (range 43–496 g/dL). Among the NF155-IgM-positive patients, CSF protein was only elevated in 1 patient; median CSF protein level was 43 g/dL (range 22–190 g/dL).

**Imaging**

Two NF155-IgG-positive patients with polyradiculoneuropathy had lumbar plexus MRIs; both showed enlarged proximal nerves and 1 also had increased T2 signal. MRI lumbar spine performed in 3 patients was unremarkable. Head MRI (n = 2) showed nonspecific T2/fluid-attenuated inversion recovery hyperintensity suggestive of microvascular changes (n = 2) or was normal (n = 2).

**NF155-IgG-Positive or IgM-Positive Nerve Biopsy Findings Were Variable**

Two sural nerve biopsies from NF155-IgG-positive cases were performed. Teased fiber preparations did not show

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**Table 2** Demographic and Clinical Comparison of NF155-IgG4, Contactin-1–IgG, and MAG-IgM Patients

| Category                          | NF155-IgG4 (n = 20) | Contactin-1–IgG (n = 9)* | p Value* | MAG-IgM (n = 44) | p Value* | NF155-IgM (n = 7) | p Value† |
|-----------------------------------|---------------------|--------------------------|----------|------------------|----------|--------------------|----------|
| Male                              | 11 (55)             | 5 (55)                   | 0.97     | 33 (75)          | 0.1      | 5 (71)             | 0.44     |
| Onset age, mean, y                | 45.5                | 63                       | 0.18     | 62.3             | <0.001   | 54                 | 0.53     |
| Follow-up, mo, mean               | 85                  | 92                       | 0.13     | 117.2            | 0.64     | 45                 | 0.1      |
| Sensory ataxia                    | 18 (90)             | 7 (78)                   | 0.37     | 28 (64.5)        | 0.08     | 0                  | —        |
| Cerebellar ataxia                 | 4 (80)              | 0                        | 0.14     | 1 (2)            | 0.01     | 0                  | —        |
| Tremor                            | 10 (50)             | 3 (33)                   | 0.4      | 14 (31)          | 0.16     | 0                  | —        |
| CISP/CISP+ phenotype              | 0                   | 4 (44)                   | 0.005    | 0                | 0        | 0                  | —        |
| Neuropathic pain                  | 9 (45)              | 4 (44)                   | 0.97     | 17 (56)          | <0.001   | 2                  | 1        |
| Monoclonal protein                | 1 (5)               | 1 (11)                   | 0.34     | 48 (100)         | <0.001   | 0                  | —        |
| Glomerulonephritis                | 0                   | 2 (22)                   | 0.08     | 0                | 0        | 0                  | —        |
| CSF protein                       | 224 (60–834)        | 87.5 (50–960)            | 0.31     | 68 (44–152)      | 0.01     | 43 (22–190)        | 0.01     |
| Root enhancement on MRI           | 14 (82)             | 4 (50)                   | 0.09     | 3 (27)           | <0.001   | 0                  | —        |
| Treated with IVIg†                 | 18 (90)             | 8 (89)                   | 0.92     | 8 (18)           | <0.001   | 5                  | 0.26     |
| Treated with rituximab§            | 5 (25)              | 4 (44)                   | 0.29     | 4 (9)            | 0.08     | 0                  | —        |
| Initial response to IVIg¶         | 14 (70)             | 6 (75)                   | 0.66     | 0*               | <0.001   | 3                  | 0.2      |
| Response to rituximab¶            | 4 (80)              | 3 (75)                   | 0.95     | 0*               | 0.01     | 0                  | —        |
| INCAT first visit                 | 5 (1–8)             | 2 (2–9)                  | 0.11     | 1 (0–3)          | <0.001   | 3 (0–3)            | <0.001   |
| INCAT last visit                  | 2.5 (1–7)           | 3 (0–9)                  | 0.04     | 3 (0–4)          | 0.51     | 2.5 (1–3)          | 0.48     |

Abbreviations: CISP = chronic immune sensory polyradiculopathy; IgG = immunoglobulin G; IgG4 = immunoglobulin G4; IgM = immunoglobulin M; INCAT = Inflammatory Neuropathy Cause and Treatment; IV Ig = IV immunoglobulin; MAG = myelin-associated glycoprotein; NF155 = neurofascin-155. Values are n (%) or median (range) unless otherwise specified.

* All 9 patients were seen at Mayo Clinic; 7 with serum available were contactin-1–IgG4 positive.

§ Imaging available in NF155-IgG4 (n = 17), contactin-1–IgG (n = 8), and MAG-IgM (n = 11).

§ One additional contactin-1 patient was treated with IVMP.

¶ Modified Rankin Scale ≥1, INCAT ≥1 for longer than 3 months.

* One patient was stable on IVIg and on rituximab.

† Treatment response was available in 3/5 patients with IVIg treatment.

§ Statistical comparison between cases with NF155-IgG4 and cases with Contactin-1-IgG.

§ Statistical comparison between cases with NF155-IgG4 and cases with MAG-IgM.

§ Statistical comparison between cases with NF155-IgG4 and cases with NF155-IgM.

Among the NF155-IgM-positive patients, electrodiagnostic findings were length-dependent axonal polyneuropathy (n = 3), mixed axonal and demyelinating polyneuropathy (n = 2), upper trunk brachial plexopathy (n = 1), and normal (n = 1). Blink studies were normal in all tested patients (n = 5).

Among all 5 NF155-IgM-positive cases, MRI lumbar spine was available for review. One patient showed nonenhancing T2 hyperintensity. MRI lumbosacral plexus was abnormal in 2 of the 4 patients showing T2 hyperintensities of the proximal nerves without gadolinium enhancement. Head MRI brain showed nonspecific T2 hyperintensities (n = 2) or was normal (n = 2).
First-line treatments included IVlg (n = 4) and PLEX (n = 1). Two patients needed additional long-term immunosuppression (azathioprine and mycophenolate mofetil).

Among NF155-IgG-positive cases (n = 7), 6 were treated with immunotherapy. One patient who had facial numbness and borderline normal neurologic examination was not treated. Treatment included IVlg (n = 5) or IVMP (n = 1).

Among the NF155-IgG-positive cases, median follow-up time was 7 years (range 52–253 months). As a group, all had favorable outcome. One patient was only treated for pain (diagnosis length-dependent large fiber sensory peripheral neuropathy). Gait aids were utilized at presentation in 2 patients (walker and wheelchair) and these 2 patients continued to require a walker at last follow-up.

Among the NF155-IgM-positive cases, median follow-up time was 4 years (range 2–128 months). Treatment response evaluation was available in 5 NF155-IgM-positive cases who had neuromuscular clinic follow-ups. Four cases significantly improved with treatment. One patient with brachial plexopathy did not demonstrate muscle strength improvement but his neuropathic pain significantly improved. Gait aids at presentation were needed in 4 patients (cane = 3, wheelchair = 1) and at last follow-up 2 patients still required a cane.

Class of Evidence
This study provides Class III evidence that NF155-IgG4-seropositive patients, compared to typical CIDP, present with distal more than proximal muscle weakness, positive sensory symptoms, and gait ataxia.

Discussion
The occurrence of NF155 IgG4 seropositivity in our US-based tertiary center for patients with immune-mediated demyelinating neuropathies was 6.5%. This is similar to other studies, which reported a frequency of 4%–18%.1,4,8 Serologically, NF155 autoantibodies observed in this study were mainly IgG4 subtype, with a distinct clinical pathologic phenotype. Although a considerable proportion of NF155-IgG-positive or NF155-IgG-positive patients who were NF155-IgG4-negative had electrophysiologic, radiologic, and histopathologic findings suggestive of an immune or inflammatory neuropathy, but no consistent clinical phenotype, histopathologic findings or treatment response was observed (Table 2). More specifically, compared to NF155-IgG4-positive patients, there was significantly lower CSF protein (p = 0.01) and INCAT score at presentation (p < 0.001). This was also shown by other groups, with few patients with chronic indolent idiopathic or genetic neuropathies testing positive for NF155-IgG/NF155-IgM antibodies.19 One hypothesis is that there is a transient IgM or IgG1-3 humoral response to NF155 antigens among neuropathies of varied etiologies that may be secondary to paronal damage and antigenic release.20
Our study shows high frequency of neuropathic pain and autonomic symptoms (mainly sudomotor dysfunction, 45%) in NF155-IgG4-positive patients, which clinically suggests small fiber involvement in this disease. Median CASS scores were higher than described in classic CIDP.\textsuperscript{21} Small fiber involvement was also a part of the pathologic abnormalities seen on electron microscopy, showing nonmyelinating Schwann cell cytoplasm infiltrated by bundles of collagen fibers instead of axons, so-called “collagen pockets” (Figure 1G). This indicates that in addition to more distal pattern of muscle weakness and sensory ataxia, autonomic involvement or pain as initial symptom should raise a concern for NF155-IgG4 positivity.\textsuperscript{21} Presence of papilledema and demyelinating features on electrodiagnostic testing with only a minority of studies demonstrating conduction block suggests that NF155-IgG4 testing should also be considered among patients clinically suspected to have POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes).

Blink reflex latency measurements as a reflection of subclinical demyelination were recently reported to be prolonged in a small cohort of NF155-IgG4-positive patients.\textsuperscript{22} Our findings agree with this observation and we show that the majority of our patients (84%) had prolonged blink responses. This can be a useful tool to detect proximal demyelination in the presence of severe axonal degeneration in distal nerves, which is encountered in many NF155-IgG4-positive patients.

Fascicular sciatic biopsy taken from a severely affected patient with sensory ataxia and tremor demonstrated diffuse loss of myelinated fibers, thickened perineurium, and a large degree of subperineurial edema, similar to what has been previously shown in distal sensory NF155 nerves (Figure 2A).\textsuperscript{9} What has not been previously shown were the high frequency of segmental demyelination (48%) and associated large epineurial perivasculat immediate inflammatory collections supportive of proximal inflammatory pathology (Figure 2C). Pathologically, more severe neurologic involvement in a proximal fascicular sciatic biopsy than in distal cutaneous nerve biopsies of NF55-IgG4-positive patients is similar to what is seen in classical CIDP.\textsuperscript{23} We also show various degrees of paranodal swellings (myelin thickening) on teased nerve fibers from proximal and distal biopsies among NF55-IgG4-positive patients. The demyelination on NCS and hypotrophy of nerves on MRI made us expect biopsy specimens would show typical hypertrophic features of onion bulb formation, but those were absent in NF155-IgG4-positive cases but present in an NF155-IgG-positive patient (Figure 2H), highlighting that distinct histopathologic changes may be limited to NF155-IgG4-positive cases, and NF155-IgG-positive or NF155-IgM-positive nerve biopsy findings may vary based on associated neuropathy phenotype.

We compared the clinical features of other seropositive demyelinating polyradiculoneuropathies to NF155-IgG4-positive patients (Table 2). Younger age at neuropathy onset, sensory ataxia, cerebellar signs, and neuropathic pain at onset were more common among NF155-IgG4-positive patients in comparison to MAG-IgM-positive distal neuropathy. Presenting features sensory ataxia and neuropathic pain were common among NF155-IgG4 and contactin-1–IgG-seropositive patients, making clinical distinction between these 2 conditions during initial presentation difficult. However, 4 of the 9 contactin-1 cases had a CISP or CISP-plus phenotype\textsuperscript{17,30} and 2 of the 9 cases had membranous glomerulonephropathy versus none of the NF155-IgG4-positive patients. Disease progression among NF155-IgG4 and contactin-1 IgG tends to be more rapid than in MAG-IgM, which has an insidious progression. Furthermore, both NF155-IgG4 and contactin-1 IgG cases were more immunotherapy responsive in comparison to MAG-IgM-associated neuropathy (Figure 5). Comparing neurologic outcomes between NF155-IgG4 and contactin-1–IgG-seropositive patients showed that the latter had a trend towards a more aggressive clinical course.
A limitation of our study is its retrospective nature. The data collected are not population-based; therefore, the frequency of NF155 may be skewed. Evaluation of assay clinical specificity during assay validation is critical; these data are not readily available for antibody evaluations performed at outside laboratories. As has been demonstrated in the past for other cell surface antibodies, ELISAs may generate a higher number of false-positive results. Therefore, multicenter studies comparing assay metrics of the different testing methodologies utilized for detection of NF155 autoantibodies should be performed.

NF155-IgG4 is a highly specific biomarker of clinically, electrophysiologically, and histopathologically distinct demyelinating polyradiculoneuropathy. However, the clinical utility of NF155-IgM or NF155-IgG without coexisting NF155-IgG4 remains unclear. Therefore, NF155-IgG-positive/NF155-IgM-positive patients should be managed only on the basis of their clinical and electrodagnostic phenotype. The clinical course and outcome are different from typical CIDP, with most patients requiring long-term immune treatment (<2 years) and the utilization of more aggressive immunotherapy.

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