

RESEARCH ARTICLE

Clinical and diagnostic features of anti-neurofascin-155 antibody-positive neuropathy in Han Chinese

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Abstract

Objective: To investigate the clinical features of Han Chinese patients with anti-neurofascin-155 (NF155) antibody-positive neuropathy. **Methods:** We screened 194 patients with peripheral neuropathy for NF155 antibodies using a cell-based assay (CBA) and teased-fiber immunofluorescence assay. We summarized the clinical findings of seropositive patients. **Results:** The sera from 17 patients reacted to human embryonic kidney 293 cells transfected with NF155. Eleven of these patients had the immunoglobulin G (IgG) 4 isotype, a younger onset age, tremor, higher levels of cerebrospinal fluid protein, a larger diameter of the lumbosacral nerve root on magnetic resonance imaging, and the distal demyelinating symmetric phenotype. Most patients responded to steroids and rituximab. For the remaining six seropositive patients in CBA, the predominant antibody isotype was IgG3, IgG1, or undetectable, and only one patient with IgG3 showed a positive result in the teased-fiber immunofluorescence assay. These patients did not share the typical features displayed by patients with the IgG4 isotype. **Interpretation:** In the Han Chinese population, a significant proportion of patients who fulfilled the criteria for chronic inflammatory demyelinating polyradiculoneuropathy diagnosis had anti-NF155 IgG4 antibody-positive neuropathy and displayed specific phenotypes. Ambiguous staining patterns may appear, and the potential for false positivity should be considered. For patients who presented with specific phenotypes, identifying antibodies and subtypes involved a significant laboratory workup.

Introduction

Recent studies have revealed antibodies against proteins in the nodes or paranodes of Ranvier in patients fulfilling the 2010 European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) criteria for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) with specific features. Neurofascin-155 (NF155) is located in the paranode region of Ranvier and Schmidt–Lanterman incisures.¹ It acts as the glial ligand of contactin-1 (CNTN1) and contactin-associated protein-1 (CASPR1) to form a paranodal complex separating the node and internode regions. The complex has a significant role in the aggregation and stabilization of Nav

channels.² Since Pruss et al.³ first proposed NF155 as a new immunological target of antibodies in autoimmune peripheral neuropathy, a series of studies have described specific features in patients with anti-NF155 antibodies,^{4,5} including tremor, sensory ataxia, extremely elevated cerebrospinal fluid (CSF) protein levels, and a poor response to intravenous immune globulin (IVIg) in patients with immunoglobulin (IgG)4 as the predominant isotype. The pathogenic effects of NF155 deficiency have been investigated that the genetic deletion of NF155 injures the septate-like junctions. Passive transfer experiments also demonstrated that the antibodies can reduce the formation of the paranodal complex and alter the conduction of motor nerves.⁶ However, few systematic clinical studies

have been conducted in China. In this study, we assessed the isotypes of antibodies against NF155 in our cohort of patients with peripheral neuropathy in the Han Chinese population. We also summarized the clinical, neuroimaging, and electrophysiological features of the seropositive patients.

Patients and Methods

Patients and samples

One hundred ninety-four patients with peripheral neuropathy who were admitted to Qilu Hospital (Jinan City, China) from March 2018 to November 2019 were enrolled in the study. The patients had CIDP ($n = 46$), Guillain–Barre syndrome (GBS) ($n = 83$), and other peripheral neuropathies (e.g., amyloidotic peripheral neuropathy, hereditary peripheral neuropathy, multifocal motor neuropathy, and undiagnosed peripheral neuropathy) ($n = 65$). All CIDP patients fulfilled the definite electrodiagnostic criteria for CIDP based on the EFNS/PNS Guideline criteria (2010) as diagnosed by two experienced neurologists. Clinical and electrophysiological data were collected. Informed consent was obtained from all patients. The study was approved by the Ethics Committee of Qilu Hospital, Shandong University, China (KYL-2021(KS)-985).

Cell-based assay

Human embryonic kidney 293 cells were plated in 24-well plates with fetal bovine serum-supplemented Dulbecco's modified Eagle's medium (DMEM) and incubated for 24 hours. Cells were transfected with a green fluorescent protein (GFP)-marked expression vector containing cDNA encoding human NF155 NM_(001160331) obtained from the Department of Neuroimmunology, Henan Institute of Medical and Pharmaceutical Science, Academy of Medical Science, Zhengzhou University (Henan Province, China) at 70%–80% confluence using lipo2000 (Invitrogen; Waltham, MA, USA). The GFP tag was located in the C-terminal portion. Four hours after transfection, the culture medium was replaced with DMEM and 10% fetal bovine serum. Transfected cells were incubated for another 24–36 h.

Cells expressing GFP-NF155 were fixed, permeabilized, and blocked before incubation with diluted sera (1:50) in phosphate-buffered saline (PBS) for 2 h at 37°C. The cells were subsequently treated for 45 min with Alexa Fluor 594-goat anti-human IgG Fc γ (1,1000; Jackson ImmunoResearch, West Grove, PA, USA) and rinsed with PBS three times. The results were visualized under a fluorescence microscope (Leica, Solms, Germany).

Immunofluorescence assay on teased fibers

Teased fibers were dissected from the sciatic nerves of adult C57BL/6J mice on adhesion microscope slides and fixed in acetone at room temperature for 10 min. The slides were permeabilized with 1% Triton X-100 at 37°C for 30 min, blocked, and incubated with sera diluted at 1:10 with PBS together with chicken anti-human/mouse/rat neurofascin antigen affinity-purified polyclonal antibodies (1:50; R&D Systems, Minneapolis, MN, USA) overnight at 4°C, and then incubated with AffiniPure goat anti-human IgG Fc γ (1:200; Alexa Fluor 594; Jackson ImmunoResearch) together with AffiniPure goat anti-chicken IgY (IgG) (H + L) (1:200; Alexa Fluor 488; Jackson ImmunoResearch) at 37°C for 45 min. Images were acquired using a fluorescence microscope (Leica).

IgG subclasses

After incubating transfected cells with diluted sera, the cells were labeled with mouse antibodies against human immunoglobulin G1 (IgG1) (1:200; Thermo Fisher, Waltham, MA, USA), immunoglobulin G2 (IgG2) (1:200; Thermo Fisher), immunoglobulin G3 (IgG3) (1:200; Thermo Fisher), or immunoglobulin G4 (IgG4) (1:200; Thermo Fisher) for 2 h at 37°C, followed by Alexa Fluor 594-conjugated goat anti-mouse IgG (H + L) preadsorbed antibodies (1:500; Abcam, Cambridge, United Kingdom) for 40 min.

Clinical, neuroimaging, and electrophysiological data analyses

To evaluate the clinical divergence of patients bearing anti-NF155 antibodies with different isotypes in CBA, we compared the seropositive patients with the seronegative patients in our study who were diagnosed with definite CIDP based on the EFNS/PNS Guideline criteria.⁷ Two additional anti-NF155 IgG4 seropositive patients who were diagnosed later were included to expand the sample size.

All patients underwent a detailed clinical examination. To evaluate response to treatments, the Hughes Disability Scale was used to assess disability in patients.⁸ We defined treatment responses in terms of Δ Hughes (the scale value after treatment minus that before treatment) as follows: Δ Hughes < 0, effective; Δ Hughes = 0, with subjective or objective improvement, partially effective; and Δ Hughes \geq 0, without any improvement, non-effective. Magnetic resonance neurography (MRN) was performed for eight patients using a 3.0 T MR scanner. Based on the lumbosacral plexus coronal maximum intensity projection

images, the diameter of the bilateral lumbar 3–sacral three nerve was measured and compared. The diameter of the lumbosacral plexus was defined as the maximum vertical length of the root on MRN images. All patients underwent electromyography (EMG). The electrophysiological characteristics involved the motor and sensory conduction of the upper and lower extremities. The distal motor latency, motor conduction velocity, and amplitude were recorded bilaterally using standard protocols. We calculated the terminal latency index (TLI) following the method previously described by Katz et al.⁹: $TLI = \text{distal distance} / (\text{proximal conduction velocity} \times \text{distal motor latency})$.

The variables were analyzed using IBM SPSS Statistics 23. Continuous variables that followed a parametric distribution were analyzed using Student's *t*-test. Nonparametric variables were analyzed using the Wilcoxon rank test. Correlations were calculated using the Pearson's coefficient. Statistical significance was set at a *p*-value of <0.05.

Results

Features of antibody reactivity

We screened all serum samples and 93 CSF samples obtained from 194 patients for anti-NF155 IgG antibodies. Fifteen patients were seropositive for the antibodies, as determined in CBA. In nine patients, IgG4 antibodies were predominant with varying degrees of IgG1 or IgG2 contribution (Fig. 1A and B). The sera of the patients showed reactivity in the paranodal regions of the teased fibers, with a staining pattern similar to that of commercial pan-neurofascin polyclonal antibodies (Fig. 1C). The positivity rate of anti-NF155 IgG4 among patients with definite CIDP was 19.6% (9/46 patients). Six patients showed seropositive for non-IgG4 antibodies against NF155 in CBA. Two patients with CIDP predominantly had the IgG1 isotype; two patients (Patient 16 with hereditary neuropathy with pressure palsies (HNPP) and Patient 13 with subacute-onset CIDP) had the IgG3 isotype; and two patients with CIDP had an undetectable isotype. Reactivity against paranode regions in the immunofluorescence assay occurred in only one CIDP patient (Patient 13; Fig. 1C), whereas no definite reactivity occurred in the remaining five patients. CSF samples from eight patients (Patients 1–5, 7, 13, and 15) were tested and were all positive, as detected by CBA. The CBA and IHC results of Patient 16 are presented in Figure S1.

To better analyze the clinical features, in addition to these nine patients, we included two other patients with anti-NF155 IgG4 (Patients 10 and 11) who had been

diagnosed after initial enrollment. The antibody isotypes of all 17 patients are presented in Table 1.

Clinical and laboratory features of patients with NF155 antibodies

Of the 11 patients with the IgG4 isotype, nine patients were male and two patients were female. The mean age of onset was 31.4 ± 17.9 years old. The average disease course to diagnosis was 9.3 months. Flu-like symptoms preceding the onset of the disease were recalled by 27.3% (3/11) of patients. Intentional or postural tremor occurred in 90.9% (10/11) of the patients, and the symptoms generally worsened during weight bearing. All (100%; 11/11) patients had sensory ataxia with a positive Romberg's sign, and 54.5% (6/11) of patients complained of difficulty maintaining balance while walking. Nine patients with the IgG4 isotype underwent a lumbar puncture, and their mean CSF protein levels were markedly higher (2.054 ± 0.97 g/L) than those of the seronegative group (1.29 ± 0.21 g/L; *p* = 0.007). In addition, we noticed that three IgG4-seropositive patients had high-arched feet, and one patient among them had severe atrophy in the lower leg muscles (Fig. 2A). Eight patients had a lumbosacral plexus on MRN. All of these patients had diffuse hypertrophy, edema, or abnormal enhancement signals of the lumbosacral plexus (Figs. 2C and D). The largest lumbosacral diameters along the L3–S3 root of patients with anti-NF155 IgG4 antibodies were significantly greater than those in seronegative patients with CIDP (Table 2). Brain MRI was conducted in five patients, and T2-weighted fluid-attenuated inversion recovery axial imaging revealed a subcortical high signal change in the right frontal lobe in Patient 11 (Fig. 2E).

Six patients with the IgG4 isotype underwent sural nerve biopsy. All of them had a slight nerve injury with different degrees of myelinated fiber loss (5%–20%) (Fig. 3A). Myelin digestion chambers were occasionally observed with modified Gomori trichrome staining (Fig. 3B). Myelin ovoids were present in two patients (Fig. 3C). In addition, one patient had evident subperineurial edema (Fig. 3D). For the ultrastructural examination, we mainly focused on longitudinal sections to access the morphology of the Ranvier nodal and paranodal zones. We observed expanded and clear spaces of different degrees between the axon and the paranodal loops in two patients (Figure S2).

Of the other six non-IgG4 seropositive patients based on CBA, five patients fulfilled the EFNS/PNS Guideline criteria for definite CIDP. The remaining patient was diagnosed with HNPP and had a large fragment deletion of the *PMP22* gene. The key clinical features of IgG4-seropositive patients, non-IgG4-seropositive CIDP

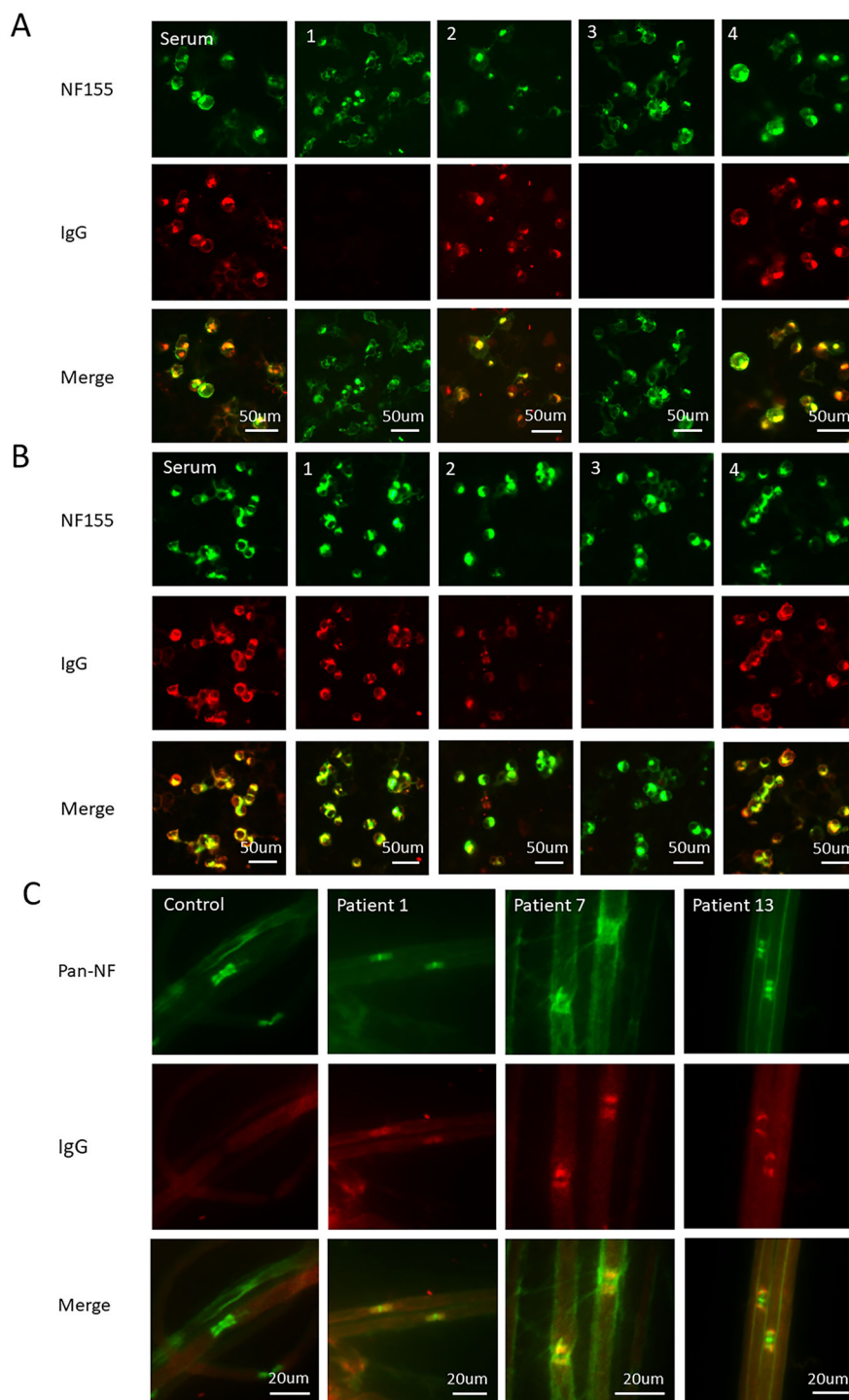


Figure 1. CBA and teased nerve fiber immunofluorescence results. (A) In Patient 7, IgG2 and IgG4 results are positive, whereas IgG1 and IgG3 results are negative. (B) In Patient 6, anti-NF155 IgG antibody is detected in the serum by CBA. IgG isotype detection indicates positive results for IgG1, IgG2, and IgG4, but negative results for IgG3. (C) Double immunostaining of teased nerve fibers with sera from healthy controls and Patients 1, 7, and 13. Optimal colocalization is noted in Patients 1, 7, and 13 in the paranodal region and not in the control group. CBA, cell-based assay; IgG1/2/3/4, immunoglobulin G1/2/3/4; NF155, neurofascin-155.

Table 1. Antibody isoforms of 17 seropositive patients in CBA.

No.	Serum (CBA)					CSF (CBA) IgG	Serum (IHC) IgG	Final diagnosis
	IgG1	IgG2	IgG3	IgG4	Undetectable			
1	-	-	-	+	-	+	+	CIDP
2	-	-	-	+	-	+	+	CIDP
3	-	+	-	+	-	+	+	CIDP
4	-	+	-	+	-	+	+	CIDP
5	-	-	-	+	-	+	+	CIDP
6	+	+	-	+	-	NA	+	CIDP
7	-	+	-	+	-	+	+	CIDP
8	-	+	-	+	-	NA	+	CIDP
9	-	-	-	+	-	NA	+	CIDP
10	-	-	-	+	-	NA	+	CIDP
11	-	-	-	+	-	NA	+	CIDP
12	-	-	-	-	+	NA	-	CIDP
13	-	-	+	-	-	+	+	CIDP
14	-	-	-	-	+	-	-	CIDP
15	+	-	-	-	-	+	-	CIDP
16	-	-	+	-	-	NA	-	HNPP
17	+	-	-	-	-	NA	-	CIDP

CBA, cell-based assay; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; HNPP, hereditary neuropathy with pressure palsies; CSF, cerebrospinal fluid; IgG1/2/3/4, immunoglobulin G1/2/3/4; NA, not available.

The symbol "+" indicates a positive result (i.e., antibodies are present) and "-" indicates a negative result (i.e., antibodies are not present).

patients (Patient 16 with HNPP was not included), and seronegative CIDP patients are compared in Table 3. More than one-half of the seropositive patients exhibited distal acquired demyelinating symmetric neuropathy (DADS), despite having different IgG isotypes, whereas most seronegative patients had typical CIDP. Patients with non-IgG4 against NF155 did not have the characteristics of younger onset age, ataxia, disturbance of deep sensation, and higher CSF protein levels, as found in patients with the IgG4 isotype. However, trembling seemed more common in these patients than in seronegative CIDP patients.

Electrophysiological study

In the motor nerve conduction study, a significantly longer distal latency (DL) and smaller TLI in the median, ulnar, and tibial nerves were observed in patients with anti-NF155 IgG4 antibodies. Prolonged DL was observed in the peroneal nerve, but TLI did not differ significantly from that in the seronegative group. The compound muscle action potential amplitude of the tibial nerve was lower in the IgG4 seropositive group, which indicated axonal damage and more severe involvement of the lower limbs. The non-IgG4 seropositive group had a smaller TLI only in the tibial nerve but did not show any evidence of distal dominant demyelination. Motor conduction velocity in both groups did not differ significantly from that in the seronegative group. In sensory nerve

conduction, elicited sensory nerve action potentials were fewer in IgG4-seropositive patients, which demonstrated severe sensory nerve involvement. However, similar features were not observed in the non-IgG4 group. Detailed electrophysiological data are provided in Table 4.

Treatment

The treatments and responses are listed in Table 5. Among the 17 seropositive patients, four patients were treated with IVIg. A marked improvement occurred only in one (25%) patient with subacute-onset CIDP with anti-NF155 IgG3 antibody (Patient 13), whereas the other three IgG4-seropositive patients had a poor response to IVIg. Fourteen patients were treated with steroids, and 12 (85.7%) patients improved to various degrees. We noted that Patient 11, who had a recurrent course, had a marked response to 500 mg methylprednisolone at the first attack. Similar symptoms appeared again 5 days after the rapid tapering of oral prednisone. She experienced a limited effect after steroid pulse therapy with the second attack. The patient was then treated with rituximab and showed improvement. Patient 9 also had a similar relapsing course during the decrement of prednisone. Three patients were treated with methotrexate, and a partial improvement occurred in only one (33.3%) patient. Two patients who showed unsatisfactory responses to steroid treatment showed better responses to plasma exchange. Five patients were treated with rituximab, and all (100%)

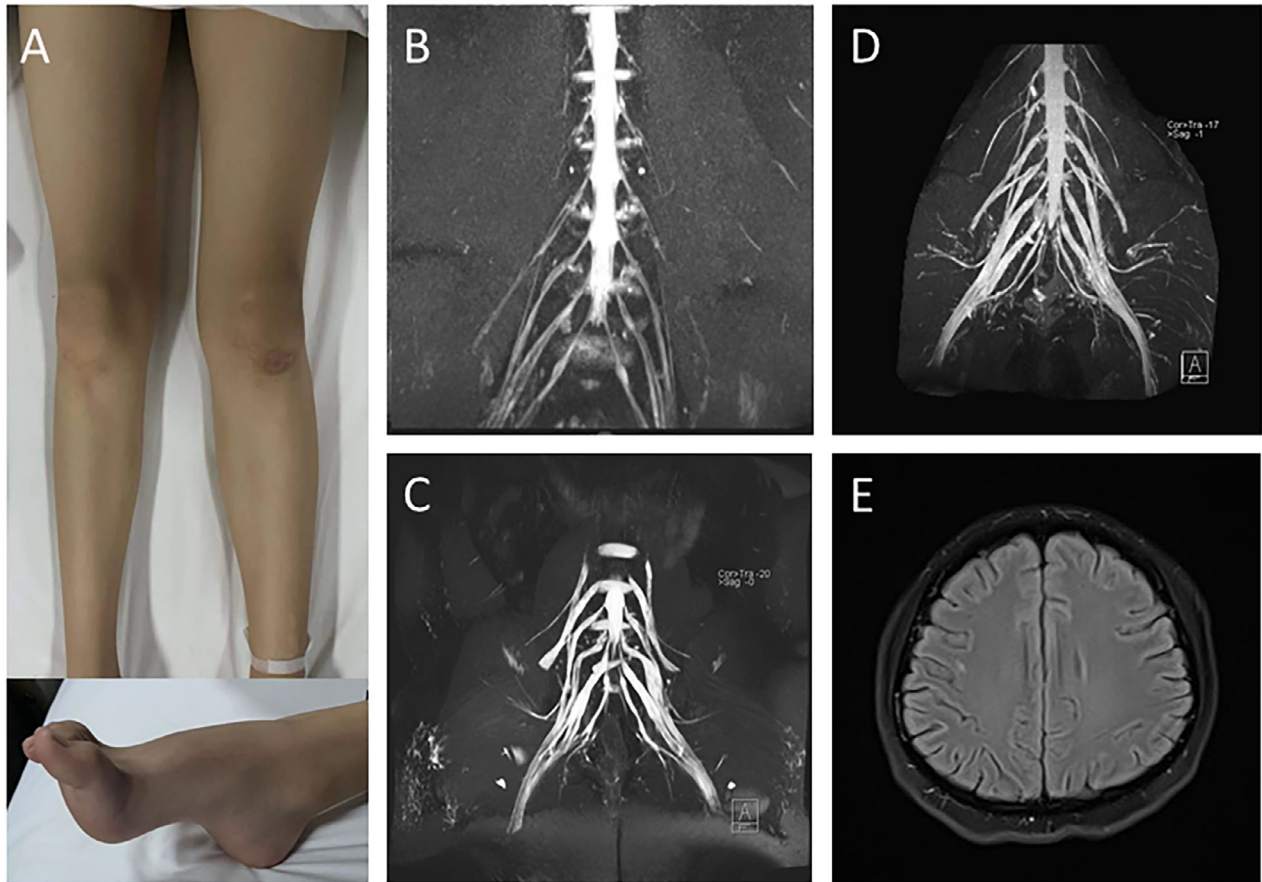


Figure 2. High-arched foot in Patient 7 and magnetic resonance imaging (MRI) findings. (A) Patient 7 presents with the typical arched foot, hammer toes, and atrophy in the lower leg muscles. (B) Lumbo-sacral plexus MRI of a control subject. (C-D) MRI of the lumbo-sacral nerve roots in Patients 2 and 11, respectively, reveals thickening of the lumbo-sacral nerve. (E) Cranial MRI of Patient 11 shows a small patch of equal T1 and a slightly long abnormal T2 signal in the right frontal lobe.

Table 2. Diameter of the lumbo-sacral nerve roots in IgG4 seropositive and seronegative CIDP patients.

Lumbo-sacral nerve root	Diameter (cm)		p-value
	IgG4- seropositive	Seronegative CIDP	
L3	0.63 ± 0.097	0.41 ± 0.145	0
L4	0.68 ± 0.129	0.49 ± 0.185	0.001
L5	0.87 ± 0.168	0.59 ± 0.265	0
S1	0.78 ± 0.104	0.59 ± 0.246	0.004
S2	0.57 ± 0.172	0.46 ± 0.253	0.016
S3	0.30 ± 0.091	0.30 ± 0.214	0.158

CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; IgG4, immunoglobulin G4. The numbers in bold font indicate a significant difference.

of these patients displayed significant improvement in the neurological and electrophysiological studies. Serum samples collected from three patients with anti-NF155 IgG4

antibodies at 3 months after clinical symptom mitigation yielded negative results.

Discussion

Newly discovered antibodies against nodal-paranodal cell adhesion molecules have been considered biomarkers associated with CIDP pathogenesis. However, in a recently published guideline,¹⁰ neuropathies with these antibodies are no longer classified as CIDP, having been reclassified as autoimmune nodopathies based on their distinct clinical features. In our study, we analyzed the clinical features of 17 patients with peripheral neuropathy in the Han Chinese population who were seropositive against neurofascin-155 in CBA. These patients included 11 patients with the IgG4 isotype predominantly and six patients with non-IgG4 isotypes. Based on previous studies,^{4,11-13} the frequency of CIDP cases with antibodies against NF155 ranges from 3.6% to 18%. The discrepancies among studies may be

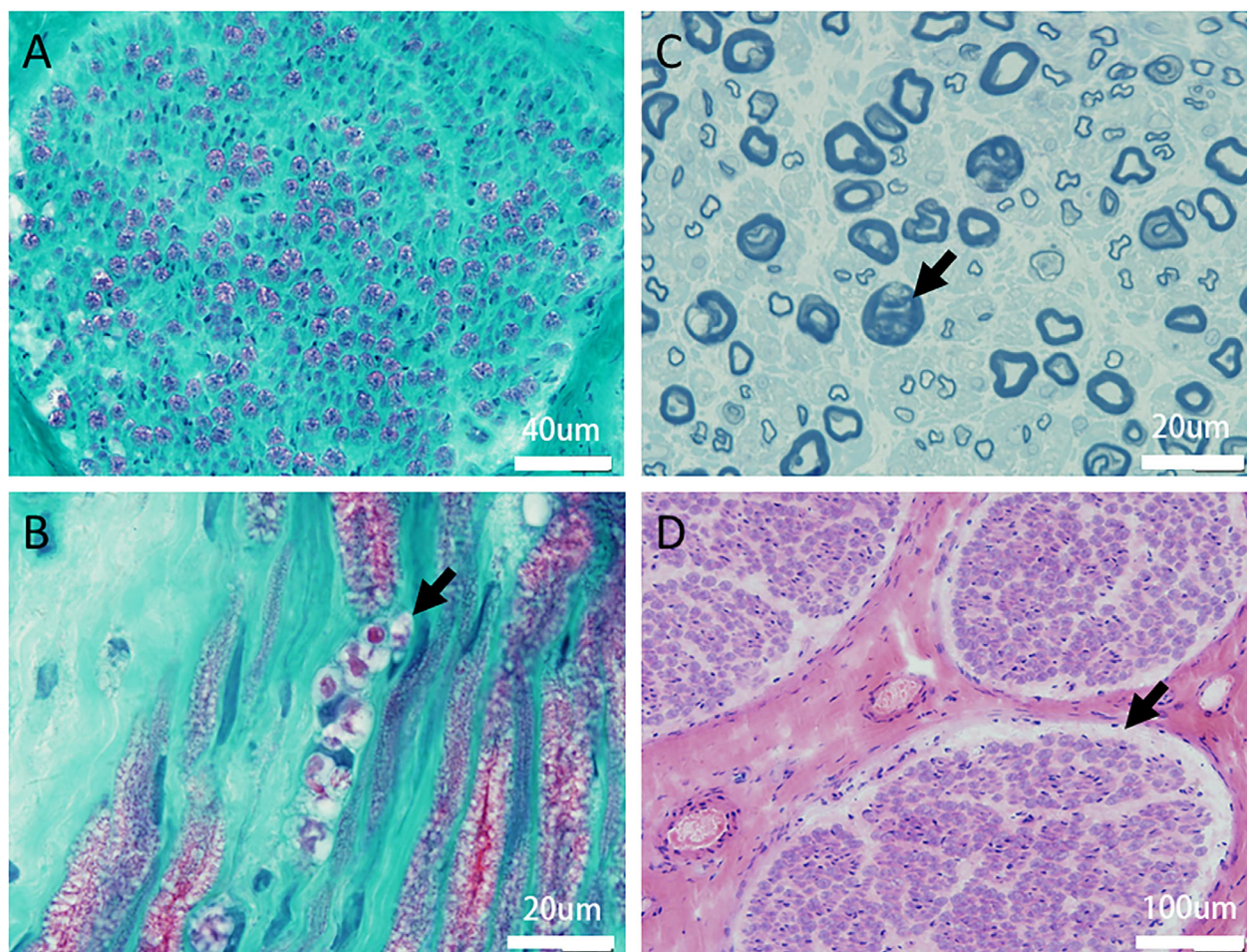


Figure 3. Nerve biopsy findings. (A) The myelinated fibers show slight loss of myelinated fiber of approximately 20% (Patient 5; MGT, magnification $\times 400$). (B) A myelin digestion chamber (Patient 1; MGT, $\times 1000$). (C) Myelin ovoids (Patient 1; semithin toluidine blue, $\times 1000$). (D) Subperineurial edema (Patient 2; HE, $\times 200$). MGT, modified Gomori trichrome stain; HE, hematoxylin–eosin stain.

related to differences in their inclusion criteria. We had a high proportion (19.6%) of CIDP cases, with all enrolled patients with CIDP fulfilling the definite electrodiagnostic criteria for CIDP. We assume that the high proportion might be related to our strict inclusion criteria. Ogata et al. used the same inclusion criteria and declared a similar positive rate of 18%.¹² Zhang et al.¹⁴ first demonstrated a high frequency of 21% in China. However, drawing definite conclusions is difficult because of the small number of included patients. The difference in incidence may also be attributed to the different immunogenetic backgrounds among ethnic groups.¹² It has been reported that specific human leukocyte antigen (HLA) class II alleles possibly play a role in the production of anti-NF155 IgG4 antibodies.^{15,16}

In our cohort, anti-NF155 IgG4 antibodies were found in patients with DADS and typical CIDP. These patients presented with characteristic features that are consistent

with those reported in other countries: younger onset, tremor, sensory ataxia, enlarged nerve roots, and markedly elevated CSF protein levels. The electrophysiological results, including distal demyelination and severe lower limb involvement, were in accordance with the finding of a previous study.¹² Sensory nerve injury of the upper and lower limbs was more severe in IgG4-seropositive patients, which may account for sensory ataxia in these patients. High-arched feet were generally regarded as a characteristic associated with inherited peripheral neuropathy and were observed in three anti-NF155 IgG4-seropositive patients in our cohort. All three patients had the disease for >6 months. Tibial motor amplitude failed to be elicited in two patients and the other patient had an extremely low tibial motor amplitude. We hypothesized that this finding was associated with the degree of tibial nerve injury and the chronic course of the disease.

Table 3. Clinical features of seropositive and seronegative CIDP patients in CBA.

	IgG4-seropositive	Non-IgG4-positive	Seronegative CIDP
Demographics			
Sex ratio (M:F)	9:2	3:2	17:15
Average age at onset (y)	31.4	47.8	42.0
Clinical phenotype			
Typical	5/11 (45.5%)*	2/5 (40%)*	25/32 (78.5%)
DADS	6/11 (54.5%)*	3/5 (60%)*	4/32 (12.5%)
Focal	0	0	0
MADSAM	0	0	3/32 (9%)
Pure sensory	0	0	0
Pure motor	0	0	0
Course			
Triggering infection	4/11 (36.4%)	3/5 (60%)*	6/32 (18.8%)
Acute/subacute	0	1/5 (25%)	0
Chronic	9/11 (81.8%)	4/5 (80%)	28/32 (87.5%)
Relapsing	2/11 (18.1)	0	4/32 (12.5%)
Symptoms and signs			
Cranial nerve involvement	5/11 (54.5%)*	0	4/32 (12.5%)
Limb weakness	11/11 (100%)	5/5 (100%)	32/32 (100%)
Tremor	10/11 (90.9%)*	2/5 (40%)*	2/32 (6.3%)
Disturbance of superficial sensation	11/11 (100%)*	3/5 (60%)	20/32 (62.5%)
Disturbance of deep sensation	10/11 (90.9%)*	2/5 (40%)	12/32 (37.5%)
Ataxia	11/11 (100%)*	2/5 (40%)	10/32 (31.3%)
Electrophysiological study			
Patients with at least 2 TLI <0.25	6/11 (54.5%)*	3/5 (60%)*	4/32 (12.5%)
Conduction block	4/11 (36.4%)	3/5 (60%)	20/32 (62.5%)
CSF examination			
CSF protein (g/L)	2.05 ± 0.97*	1.09 ± 0.59	1.29 ± 0.21
CFS IgG (mg/L)	219.66 ± 73.85*	164.96 ± 129.46	146.99 ± 179.09

CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CSF, cerebrospinal fluid; DADS, distal acquired demyelinating symmetric; F, female; IgG, immunoglobulin G; M, male; MADSAM, multifocal acquired demyelinating sensory and motor neuropathy; TLI, terminal latency index.

* $p < 0.05$, compared with the seronegative group.

Regarding differential diagnosis, misdiagnosis of Charcot–Marie–Tooth disease and other inherited peripheral neuropathies should be avoided.

All eight patients who underwent lumbosacral plexus MRI had evidence of lumbar spinal root hypertrophy. Abnormal enhancement of the spinal root has been reported in some, but not all, cases of Guillain–Barre syndrome and CIDP.¹⁷ Breakdown of the blood–nerve barrier, nerve edema, continuing demyelination, and remyelination may be responsible for the marked nerve root hypertrophy. The frequency of hypertrophy in the spinal root region (100% in this series) is striking. This finding is consistent with reports indicating that anti-NF155 IgG4 antibodies prevent paranodal complex formation and aggravate demyelination. Therefore, marked hypertrophy of the nerve root disturbs CSF circulation, which partly explains the extremely high levels of CSF proteins.⁶ In cranial MRI, with the exception of a small patch of an abnormal signal in Patient 11, none of the seropositive patients had the typical radiographic

manifestations of central nervous system (CNS) demyelination. Combined central and peripheral demyelination in this study were less frequent than previously reported.¹⁸

We found the following pathological findings of anti-NF155 antibody-positive patients: moderate endoneurial edema, reduction in total number of myelinated fibers, and occasional myelin ovoids observed under light microscopy. Similar findings were reported by Koike et al.¹⁹ Sural nerve biopsy revealed mild nerve injury, which was inconsistent with the severe involvement of the lower limbs in the electrophysiological findings. The sural nerve is purely sensory and is a distal branch of the long nerve, which may produce a discord between electrophysiological and pathological findings. Typical features of anti-NF155 antibody-positive CIDP patients in the ultrastructural examination of the sural nerve include the detachment of terminal myelin loops from the axolemma,¹⁹ loss of transverse bands, and absence of classical macrophage-mediated demyelination.^{19–21} Findings of patient nerve

Table 4. Detailed electrophysiological data of seropositive and seronegative CIDP patients in CBA.

	IgG4 positive	Non-IgG4 positive	Seronegative CIDP	<i>p</i> value [†]	<i>p</i> ' value [‡]
MOTOR					
Median nerve	16/16 (100%)	10/10 (100%)	40/42 (95.2%)	0.374	0.482
DL, ms	8.1 ± 1.4	6.5 ± 1.7	6.7 ± 4.4	0.028	0.676
CV, m/s	34.9 ± 8.6	52.0 ± 12.8	36.5 ± 14.3	0.510	0.036
CMAP, mV	4.9 ± 2.3	6.9 ± 2.7	4.6 ± 3.4	0.379	0.093
TLI	0.23 ± 0.06	0.23 ± 0.11	0.35 ± 0.22	0.002	0.151
Ulnar nerve	16/16 (100%)	8/8 (100%)	39/40 (97.5%)	0.523	0.651
DL, ms	6.2 ± 1.1	3.7 ± 0.3	4.2 ± 2.0	0.00	0.607
CV, m/s	33.9 ± 9.8	39.1 ± 11.8	35.9 ± 13.7	0.917	0.736
CMAP, mV	5.5 ± 2.7	6.4 ± 1.6	6.0 ± 2.5	0.138	0.799
TLI	0.26 ± 0.05	0.38 ± 0.10	0.41 ± 0.14	0.00	0.879
Peroneal nerve	10/15 (66.7%)	7/9 (77.8%)	38/49 (77.6%)	0.394	0.998
DL, ms	10.7 ± 1.8	7.4 ± 2.2	7.0 ± 4.3	0.00	0.234
CV, m/s	25.0 ± 10.9	31.9 ± 6.4	31.5 ± 10.0	0.082	0.928
CMAP, mV	1.13 ± 0.93	2.4 ± 1.7	2.4 ± 1.9	0.061	0.613
TLI	0.36 ± 0.12	0.41 ± 0.20	0.49 ± 0.26	0.082	0.380
Tibial nerve	10/14 (71.4%)	10/10 (100%)	34/41 (82.9%)	0.353	0.160
DL, ms	12.0 ± 2.4	7.7 ± 1.5	6.4 ± 2.1	0.00	0.228
CV, m/s	30.0 ± 7.7	37.6 ± 6.9	29.2 ± 8.9	0.805	0.039
CMAP, mV	1.7 ± 1.8	1.2 ± 1.7	4.4 ± 5.1	0.041	0.062
TLI	0.30 ± 0.10	0.37 ± 0.23	0.64 ± 0.28	0.00	0.009
SENSORY					
Median nerve	3/15 (20%)	6/8 (75%)	25/37 (67.6%)	0.02	0.681
SNAP, uV	0.99 ± 0.71	5.6 ± 4.2	11.1 ± 7.8	0.02	0.117
CV, m/s	26.5 ± 2.5	35.1 ± 10.2	50.1 ± 14.1	0.01	0.201
Ulnar nerve	3/14 (21.4%)	5/9 (55.6%)	28/35 (80%)	0.00	0.131
SNAP, uV	7.5 ± 7.3	8.3 ± 5.0	15.4 ± 12.9	0.256	0.448
CV, m/s	33.3 ± 17.3	34.5 ± 6.7	45.0 ± 13.1	0.180	0.361
Peroneal nerve	5/13 (38.5%)	3/3 (100%)	30/43 (69.8%)	0.041	0.261
SNAP, uV	3.6 ± 2.9	4.1 ± 1.3	8.0 ± 7.2	0.567	0.491
CV, m/s	34.2 ± 3.7	42.8 ± 3.8	41.3 ± 8.9	0.345	0.729
Sural nerve	5/16 (31.3%)	7/9 (77.8%)	30/39 (76.9%)	0.001	0.956
SNAP, uV	2.8 ± 2.5	5.7 ± 2.2	7.5 ± 6.5	0.321	0.852
CV, m/s	37.1 ± 3.4	41.8 ± 2.0	41.6 ± 9.8	0.531	0.996

CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CMAP, compound muscle action potential; CV, conduction velocity; DL, distal latency; IgG4, immunoglobulin G4; TLI, terminal latency index; SNAP, sensory nerve action potential.

[†]Based on the comparison between IgG4 seropositive and seronegative patients.

[‡]Based on the comparison between non-IgG4 seropositive and seronegative patients. The numbers in bold font indicate a significant difference.

biopsies and animal models have revealed that anti-NF155 disrupted axoglial junctions of the paranodal regions and caused conduction deterioration.²⁰

The antibody isotype test is important for the diagnosis of patients with anti-NF155 antibodies. In our cohort, IgG2 never occurred alone but was always accompanied by IgG4. IgG1 and IgG3 appeared alone with a weaker reactivity. Few studies have focused on the clinical features of patients with non-IgG4 isotypes. Cortese et al.²² described the features of six patients with IgG1, IgG3, or undetectable IgG isotypes. In our study, we also had six patients with non-IgG4 isotypes according to CBA results. We confirmed that these patients had atypical clinical or

electrophysiological features compared with patients with the IgG4 antibody, consistent with a previous observation.²² Most of these patients showed contradictory reactivities in CBA and IHC. Martin-Aguilar et al. reported that one patient with CMT showed a positive staining in CBA, which was however not confirmed by immunohistochemistry and ultimately classified as negative.²³ To avoid the risk of false positives due to using a single detection method, they used three techniques to confirm the existence of the antibodies and demonstrated that true positives were positive for all techniques. Their latest study indicated that approximately 10% of the positivity for antibodies against anti-NF155 detected by CBA in

Table 5. Response to treatment of 17 seropositive patients in CBA.

No.	IVIg	Steroids	Methotrexate	PE	Rituximab
1	Non-effective	Partial			
2		Non-effective	Non-effective	Partial	
3	Non-effective	Partial		Partial	Effective
4	Partial	Partial			
5		Effective			
6		Non-effective	Non-effective		
7		Partial			
8					
9		Partial			Partial
10		Partial			Effective
11		Effective			Partial
12		Effective			
13	Effective				
14		Partial			
15		Partial			
16					
17		Partial	Partial		Effective

IVIg, intravenous immunoglobulin; PE, plasma exchange.

patients were false positives, and untagged-NF155 plasmids should be preferentially used.²⁴ This agrees with the recently published revision of the EAN/PNS CIDP diagnostic guidelines.¹⁰ We consider that the patients in our group (including the four patients with CIDP and one patient with HNPP) with discrepant reactivities in CBA and IHC were most likely false positives, as described by Martin-Aguilar *et al.* The phenomenon seems to be more common in patients with non-IgG4 isotypes based on our data. Confirmatory techniques providing higher test reliability are important when antibody detection is used for clinical purposes, especially in CIDP and other neuropathies with uncertain patterns. For patients with discrepant results when different techniques are used, the risk of false positives should be considered to avoid unnecessary treatments.

Based on a previous study,¹⁹ anti-NF155 IgG4-positive neuropathy is caused by humoral immune-mediated paranodal dissection, not by classical macrophage-mediated demyelination. Autoantibodies targeting cell adhesion molecules at the nodes of Ranvier are implicated in the pathogenesis. These patients show a poor response to IVIg because the isotype does not activate the complement.²⁵ Plasmapheresis and corticoids are effective.¹² Rituximab, owing to its powerful role in B cell depletion, has been proven beneficial in patients with IgG4 who are resistant to other immunotherapies.²⁶ Three IgG4 seropositive patients responded poorly to IVIg administration. They experienced remarkable improvement in clinical symptoms and function after rituximab treatment. However, Patient 13, who had the IgG3 isotype, achieved clinical remission after IVIg treatment. The response was

presumably associated with the antibody isotype. Of note, Patient 17, whose antibodies against NF155 had the IgG1 isotype, also responded well to rituximab. We propose that rituximab should not be restricted to patients with IgG4. Two patients relapsed during the course of oral prednisone dosage reduction. We suggest that, following the amelioration of the clinical symptoms, the conditions of patients should be closely monitored when receiving a tapering dose of prednisone.

Our study has several limitations. The primary limitation is the small number of patients and the lack of false-positive confirmation. For patients whose sera contained non-IgG4 antibodies according to the cell-based assay but showed no definite reactivity in the paranodal regions of the teased fibers, a third laboratory technique should be used to confirm the existence of anti-NF155 antibodies and exclude the false positives. Furthermore, we did not detect antibody titers in serum; therefore, we were unable to compare the post-therapy changes in antibody concentrations in the serum and CSF, which we believe would be a good method for the quantitative assessment of treatment effect.

In conclusion, our findings expand on the phenotypes of patients with anti-NF155 antibodies in China. We further confirmed that a significant proportion of patients who fulfilled the criteria for CIDP had anti-NF155 IgG4 antibody-positive neuropathy and displayed specific phenotypes. For patients with specific phenotypes, the presence and isotypes of antibodies against NF155 should be examined for a refined diagnosis and guidance for therapy selection. For patients who are positive for non-IgG4 isotypes in CBA and lack the features of typical phenotypes, the possibility of false positives should be examined.

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Author Contributions

WW was responsible for study conception, design, data acquisition, analysis, access and verification of the data, interpretation of the data, and drafting of the manuscript. CL, WL, and DZ were responsible for patient inclusion, acquisition of data, interpretation of the data, and revised the manuscript. YS, JZ, and JS were responsible for access and verification of the data, analysis and interpretation, and drafting of the manuscript. ZY and CY were

responsible for study conception, design, and critical revision of the manuscript. QW was responsible for study conception, design, supervision, interpretation of the data, and revision of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data Availability Statement

All data relevant to the study are either included in the article or shared at the request of any qualified investigator.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The CBA and teased nerve fiber immunofluorescence results of Patient 16 with HNPP. IgG anti-NF155 antibody was detected in the serum using CBA. IgG isotype detection indicates positivity for IgG3 and negativity for IgG1, IgG2, and IgG4. No definite antibody reactivity was observed in the paranodal region in the immunofluorescence assay. IgG1/2/3/4, immunoglobulin G1/2/3/4; NF155, neurofascin-155.

Figure S2. Longitudinal ultrathin sections at paranodes in patient 1 (A, B) and patient 3 (C, D). Expanded spaces of different degrees between the axon and the paranodal loops and loss of transverse bands were observed. Panels B and D are the enlargement of the rectangle part of A and C, respectively.