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Short Communication

Chronic inflammatory demyelinating polyneuropathy with anti-NF155 IgG4 in China

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ABSTRACT

Patients with chronic inflammatory demyelinating polyneuropathy (CIDP) seropositive for autoantibodies against nodal and paranodal proteins display distinct clinical presentations. We herein tested for autoantibodies against neurofascin (NF) 155, NF186, contactin-associated protein 1 and contactin-1 and investigated the autoantibody-related clinical features in 29 patients with CIDP from China. Six patients with anti-NF155 IgG4 antibodies displayed younger age of onset and poor response to intravenous immunoglobulin than seronegative patients. One patient had anti-NF186 IgG antibody and no patients had anti-contactin-associated protein 1 or anti-contactin-1 antibodies. Clinical features of CIDP patients with anti-NF155 antibodies in China were similar to those reported in other countries.

1. Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an inflammatory peripheral neuropathy characterized by chronic progressive or relapsing motor and sensory deficits (Lehmann et al., 2019). The beneficial effects of corticosteroid, intravenous immunoglobulin (IVIg) and plasma exchange (PE) support a role of autoimmune-mediated mechanisms in CIDP (Lehmann et al., 2019). Recent progress on CIDP focused on the autoantibodies against the nodal and paranodal proteins (Devaux et al., 2012; Manso et al., 2019; Querol et al., 2017). Patients with different antibodies displayed a variety of clinical presentations and response to current immunotherapy. Typically, patients with IgG4 antibodies against neurofascin (NF) 155 located on the myelin paranodal loop displayed a severe phenotype with younger onset age, disabling tremor, and poor response to the IVIg treatment (Devaux et al., 2016; Ogata et al., 2015; Querol et al., 2014). IgG4 antibodies against contactin 1 (CNTN1), a protein expressed at the axonal side in the paranodal region, were associated with the subacute onset of symptoms, sensory ataxia and good response to corticosteroids (Miura et al., 2015). Furthermore, patients with CIDP seropositive for the nodal protein, NF186, also showed subacute-onset and good response to both IVIg and corticosteroids (Delmont et al., 2017). These findings raised the importance of detecting the autoantibodies in patients with CIDP to guide treatment option. We herein firstly report the presence of antibodies to nodal and paranodal proteins in Chinese patients with CIDP and analyzed the autoantibodies-related clinical features.

2. Materials and methods

2.1. Patients and samples

A total of 29 patients fulfilling the diagnostic criteria of CIDP (Joint Task Force of the EFNS and the PNS, 2010) were included from 2015 to 2018 into this study. 22 were from Shandong province, five from Hunan province, one from Jiangsu province and one from Anhui province. Written informed consent was obtained from each participant. This study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University. For all of the participants, the serum samples before treatment were collected and stored at −80 °C until use.

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Meanwhile, age and gender matched healthy donors were included as controls. The clinical data including age of onset, sex, motor and sensory deficits, cranial nerve involvement, tremor, ataxia, cerebrospinal fluid (CSF) protein levels and response to treatment (steroids, IVIg, PE and Rituximab) were collected by two of the co-authors. The acute and subacute onset was defined as previously described (Larue et al., 2011; Ruts et al., 2010). Patients were considered responsive to the treatment if improvements in muscle weakness (one or more MRC scales) were observed within one month after the beginning of the treatment. The serum samples were tested for autoantibodies against NF155, NF186, CNTN1 and contactin-associated protein 1 (Caspr1) as described below. The subclasses of the IgG antibodies were identified by ELISA as previously described (Querol et al., 2014).

2.2. Cell-based binding assay

The plasmid constructs encoding for human CNTN1, Caspr1, myc-tagged NF155 and NF186 have been described previously (Delmont et al., 2017; Devaux et al., 2016; Miura et al., 2015). Human embryonic kidney (HEK) 293 cells cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher, MA, USA) medium containing 10% fetal bovine serum (FBS) (Thermo Fisher) and 1% penicillin-streptomycin solution were transfected with human NF155, NF186, CNTN1 or Caspr1 plasmids using lipofectamine 2000 (Thermo Fisher). After 24 h, living cells were incubated with the patient serum (1:200 diluted in DMEM), Alexa Fluor 488-conjugated goat anti-human IgG (1:500, Abcam, Cambridge, UK) or DyLight 650-conjugated goat anti-human IgG (1:500, Abcam) for 20 min at room temperature. After washing, the cells were fixed in 4% paraformaldehyde for 20 min, permeabilized with 0.3% TritonX-100 for 15 min, and then incubated with mouse monoclonal anti-Myc (1:100, Beyotime, Shanghai, China), rabbit anti-Caspr1 (1:300, Abcam) or rabbit anti-CNTN1 (1:200, Abcam) antibody at 4 °C overnight. After washing, the cells were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000, Abcam) and Alexa Fluor 568-conjugated goat anti-mouse IgG (1:1000, Abcam) for 2 h at room temperature. Finally, the cells were mounted with anti-fade Poly/Mount containing DAPI (Solarbio, Beijing, China). The image was taken using a laser confocal microscope (LSM800, Zeiss, Jena, Germany).

2.3. Teased sciatic nerve binding assay

The sciatic nerve was dissected from adult C57BL/6 J mouse and fixed in 4% paraformaldehyde for 40 min at room temperature. Then, the sciatic nerve was proceeded to tease the nerve fibers on slides using fine needles. The slides were left to air dry overnight at room temperature and kept at −20 °C until use. For the assay, teased fibers were blocked with PBS containing 10% normal goat serum and 0.3% TritonX-100 for 2 h at room temperature, and then incubated with the diluted patients' sera (1:200) and rabbit anti-Caspr1 (1:300) in PBS at 4 °C overnight. The slides were washed and incubated with DyLight 650-conjugated goat anti-human IgG (1:500, Abcam) and Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000, Abcam) for 2 h at room temperature. The slides were mounted with anti-fade Poly/Mount (Solarbio). The image was taken using the laser confocal microscope.

3. Statistics analysis

Normally distributed continuous data were presented as means (standard deviations) and compared by two sample *t*-test. The categorical variables were shown as n (%) and compared by Fisher's exact test. Analysis was performed with the SPSS 20.0 analysis software (IBM, Armonk, NY). A two-sided p value of < 0.05 was considered to be significant.

Table 1
Comparison of clinical features of patients with anti-NF155 IgG and of seronegative patients.

Characteristic	Anti-NF155 ($n = 6$)	Negative ($n = 22$)	p value
Onset age (years), mean (range)	33.8 (28–40)	47.5 (16–70)	**0.002**
Male/female	4/2	13/9	1.00
Onset Acute	1 (17)	2 (9)	0.53
Subacute	0 (0)	1 (5)	1.00
Chronic	5 (83)	19 (86)	1.00
Duration before admission (days), mean (range)	57 (5–84)	71 (7–119)	0.33
Symptoms			
Limb weakness	5 (83)	12 (55)	0.17
Sensory disturbance	1 (17)	8 (36)	0.63
Cranial nerve involvement	1 (17)	6 (27)	1.00
Tremor	1 (17)	3 (14)	1.00
Ataxia	1 (17)	4 (18)	1.00
Respiratory muscle involvement	0 (0)	2 (9)	1.00
CSF protein level (g/L), mean (range)	0.8 (0.7–1.6)	1.1 (0.1–3)	0.39
Modified Rankin score at nadir, mean (range)	2.5 (1–3)	2.7 (1–4)	0.62
MRC score at nadir, mean (range)	51.2 (48–60)	49.1 (42–60)	0.49
Good response to treatments			
Corticosteroids	4/6 (67)	20/21 (95)	0.11
Intravenous immunoglobulin	0/3 (0)	6/7 (86)	**0.03**
Plasma exchange	1/1 (100)	1/2 (50)	–
Rituximab	1/1 (100)	0 (0)	–

If not indicated, the data were shown as n (%); CSF, cerebrospinal fluid.

4. Results

The demographic and clinical features of the patients with CIDP are shown in Table 1. Six of 29 patients (21%) with CIDP had IgG antibodies against NF155 (Fig. 1-A) and one (3%) had IgG antibodies against NF186 (Fig. 1-B). All of the anti-NF155 IgG were IgG4 predominant. Twenty-two patients and all of the healthy controls were serological negative and no patients had antibodies against Caspr1 and CNTN1. There were significantly younger onset age and lower frequency of good response to IVIg treatment in patients with anti-NF155 IgG4 antibodies than in the seronegative patients. The details of patients with anti-NF155 and anti-NF186 antibodies are shown in Table 2 and supplementary table 1.

5. Discussion

We herein firstly reported patients with CIDP serology positive for anti-NF155 and NF186 antibodies in China. Worldwide, the frequency of patients with autoantibodies against the nodal or paranodal proteins displays a significant variability between the different countries (Lehmann et al., 2019). This variability may be caused by the various methods used for antibody detection. Based on the combination of cell-based and teased nerve fiber binding assay, the proportion of patients with anti-NF155 IgG4 (21%) in our study was similar to that reported in Japan (9/50, 18%) but higher than those reported in Spain (2/53, 4%) or in multi-centric study including Spain ($n = 72$), Italy ($n = 42$), France ($n = 129$) and Singapore ($n = 3$) (9/246, 4%) (Delmont et al., 2017, Ogata et al., 2015, Querol et al., 2014). Our results deserve further confirmation by large Chinese cohort studies.

Patients with CIDP in our study displayed clinical features similar to those reported in previous studies (Ogata et al., 2015; Querol et al., 2014). Chinese patients with anti-NF155 antibodies had younger onset age and showed a poor response to IVIg treatment compared to the seronegative patients. One of the confirmed action mechanisms of IVIg in autoimmune diseases is to block the complement deposition induced by autoantibodies (Yuki et al., 2011). However, the predominant subclass of anti-NF155 IgG was IgG4, which does not activate the classical

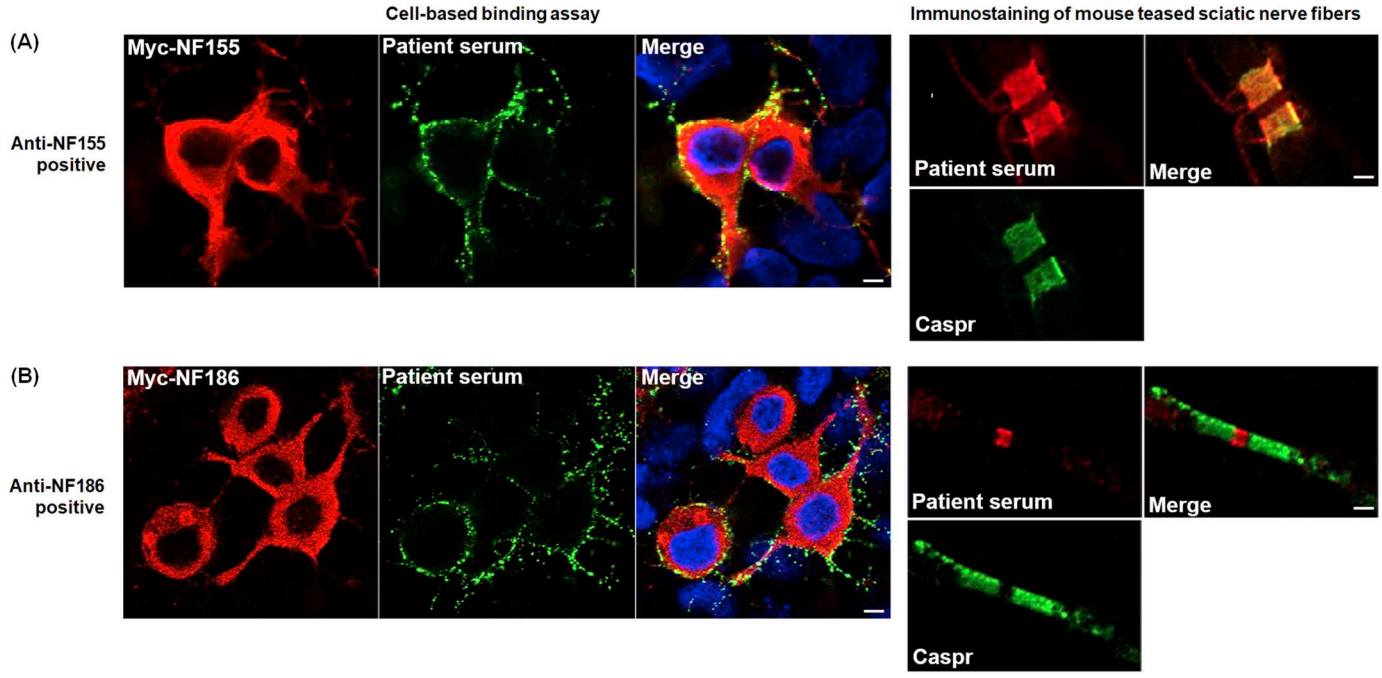

Fig. 1. Detection of anti-NF155 and anti-NF186 antibodies in patients with CIDP.
Serum samples from patients with CIDP were tested for autoantibodies against NF155, NF186, CNTN-1 and Caspr1 using cell based binding assay and teased nerve fiber binding assay. Anti-Caspr1 antibodies were used as the positive control (green) for the nerve fiber binding assay. (A) Serum from patients seropositive for anti-NF155 antibodies (green) bound specifically to HEK293 cells transfected with human NF155 plasmids and paranodal region (red) on teased nerve fibers. (B) Serum from the patient seropositive for anti-NF186 antibodies (green) bound specifically to HEK293 cells transfected with human NF186 plasmids and node of Ranvier (red) on teased nerve fibers. Nuclei were stained with DAPI (blue). Bar = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

complement pathway. Passive transfer of anti-NF155 IgG4 antibodies induced the degradation of NF155 and prevented paranodal complex formation (Manso et al., 2019). Being consistent, the recent evidence demonstrated that efficiency of IVIg in CIDP was not associated with the complement inhibition (Keller et al., 2019). These findings partly explained the poor efficiency of IVIg in seropositive CIDP. The reason why anti-NF155 antibodies are found in a younger CIDP population remains unknown. A recent study demonstrated an association between human leukocyte antigen DRB1*15 alleles and anti-NF155 positive CIDP (Martinez-Martinez et al., 2017), which could explain this younger occurrence. Further study is necessary to confirm the genetic susceptibility of anti-NF155 positive CIDP and polymorphisms of

Table 2
Clinical features of patients with anti-NF155 and anti-NF186 antibodies.

Characteristic	Anti-NF155 positive						Anti-NF186 positive
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	
Onset age/Sex	28/M	34/F	34/M	38/F	29/M	40/M	64/F
Onset	Chronic	Acute	Chronic	Chronic	Chronic	Chronic	Chronic
Duration before admission (days)	56	7	84	56	84	56	56
Clinical features							
Limbs weakness	+	−	+	+	+	+	+
Sensory dysfunction	−	+	−	−	−	−	−
Sensory ataxia	−	−	+	−	−	−	−
Cranial nerve involvement	−	−	+	−	−	−	−
Tremor	−	−	+	−	−	−	−
CSF protein level (g/L)	0.88	1.60	0.75	0.68	0.11	0.80	0.42
Brain magnetic resonance imaging	NA	−	NA	−	−	−	−
Nerve conduction study							
Demyelinating or axonal	Demyelinating	Demyelinating	Demyelinating	Demyelinating	Demyelinating	Demyelinating	Demyelinating
Axonal loss	−	−	−	−	−	+	−
Conduction blocks	−	−	+	+	−	−	+
Temporal dispersion	+	−	−	−	−	+	−
Good response to treatment							
Corticosteroids	+	−	+	+	−	+	+
Intravenous immunoglobulin	NA	−	−	NA	−	NA	+
Plasma exchange	NA	NA	+	NA	NA	NA	NA
Rituximab	NA	NA	NA	NA	+	NA	NA

M, male; F, female; CSF, cerebrospinal fluid; NA, not available.

human leukocyte antigen. One of anti-NF155 antibody related clinical feature in CIDP patients is tremor (Ogata et al., 2015), which was not confirmed in our study, possibly because of the limited cohort size. The electrophysiological and morphological findings from anti-NF155 treated animals suggested a motor predominant pathology (Manso et al., 2019). In our study, although there was no statistical difference, 5 of 6 (83%) patients with anti-NF155 antibody displayed a phenotype of predominant motor deficits, which deserve further confirmation in larger cohort studies. Similarly to previous reports (Delmont et al., 2017; Burnor et al., 2018; Stengel et al., 2019), the frequency of anti-NF186 antibodies in Chinese patients with CIDP was low and found in an elderly patient. Up to now, only eleven patients have been reported with anti-NF186 antibodies (Delmont et al., 2017; Burnor et al., 2018; Vallat et al., 2018; Stengel et al., 2019). These antibodies do not seem to have a clear association with epidemiological events, and only three patients presented with previous infection or diarrheal illness. However, bigger cohort studies are necessary to confirm this.

In conclusion, we reported a high frequency of Chinese patients with CIDP seropositive for anti-NF155 IgG4 antibody, which was associated with younger onset age and poor response to IVIg treatment.

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Declaration of competing interest

The authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.577074.

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