

REPORT**Contactin 1 IgG4 associates to chronic inflammatory demyelinating polyneuropathy with sensory ataxia****Yumako Miura,^{1,*} Jérôme J. Devaux,^{2,*} Yuki Fukami,¹ Constance Manso,² Maya Belghazi,² Anna Hiu Yi Wong¹ and Nobuhiro Yuki^{1,3} for the CNTNI-CIDP Study Group[†]**

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[†]For details of the CNTNI-CIDP Study Group see Appendix 1

A Spanish group recently reported that four patients with chronic inflammatory demyelinating polyneuropathy carrying IgG4 autoantibodies against contactin 1 showed aggressive symptom onset and poor response to intravenous immunoglobulin. We aimed to describe the clinical and serological features of Japanese chronic inflammatory demyelinating polyneuropathy patients displaying the anti-contactin 1 antibodies. Thirteen of 533 (2.4%) patients with chronic inflammatory demyelinating polyneuropathy had anti-contactin 1 IgG4 whereas neither patients from disease or normal control subjects did ($P = 0.02$). Three of 13 (23%) patients showed subacute symptom onset, but all of the patients presented with sensory ataxia. Six of 10 (60%) anti-contactin 1 antibody-positive patients had poor response to intravenous immunoglobulin, whereas 8 of 11 (73%) antibody-positive patients had good response to corticosteroids. Anti-contactin 1 IgG4 antibodies are a possible biomarker to guide treatment option.

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Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; DRG = dorsal root ganglion; GBS = Guillain-Barré syndrome

Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is clinically heterogeneous and potentially treatable

(Köller *et al.*, 2005). The most widely used treatments for CIDP consist of intravenous immunoglobulin, corticosteroids and plasma exchange, but response to each immunotherapy is variable among patients. Specific biomarkers

need to be identified to improve patient diagnosis and treatment choice.

Cell adhesion molecules play a crucial role in the formation of the nodes of Ranvier and in the rapid propagation of the nerve impulses along myelinated axons (Faivre-Sarrailh and Devaux, 2013). In the peripheral nerves, the domain organization of myelinated axons depends on specific axo-glial contacts between the axonal membrane and Schwann cells at nodes, paranodes and juxtaparanodes. Recently, we showed that some of the patients with CIDP present IgG autoantibodies directed against the nodes of Ranvier or the paranodal axo-glial apparatus (Devaux *et al.*, 2012). Notably, we identified neurofascin-186, gliomedin and contactin 1 (CNTN1) as the targets of autoantibodies in some patients with CIDP. IgG4 autoantibodies to CNTN1 were also identified in a subgroup of Spanish patients with CIDP sharing common clinical features, including aggressive symptom onset and poor response to intravenous immunoglobulin (Querol *et al.*, 2013; Labasque *et al.*, 2014).

CNTN1 is a key axonal adhesion molecule, which interacts with CNTNAP1 (previously known as Caspr1) on the axon and neurofascin-155 on the glial side (Peles *et al.*, 1997; Tait *et al.*, 2000), and is essential for the formation of the paranodal septate-like junction (Boyle *et al.*, 2001). Mice deficient in CNTN1 show paranodal alterations associated with conduction slowing (Boyle *et al.*, 2001), suggesting that the immune attack against CNTN1 has pathogenic effects. Here we investigated the target antigens in a large cohort of patients with CIDP. We describe the clinical and serological features of 13 Japanese patients with CIDP and anti-CNTN1 IgG4 antibodies. We found that anti-CNTN1 IgG4 antibodies are associated with a subset of patients with CIDP and correlated with a specific response to treatments.

Materials and methods

Patients and sera

Sera from 533 patients with CIDP, who were admitted to various hospitals in Japan and were treated naïve during the time of diagnosis and sera collection, were sent to the neuroimmunological laboratory at Dokkyo Medical University, Tochigi, Japan between 1996 and 2014 and stored at -80°C until use. Sera from 200 patients with Guillain-Barré syndrome (GBS) and 100 patients with multiple sclerosis were used as disease controls as well as sera from 100 healthy subjects as normal controls. Clinical information of each patient was obtained at admission, discharge and follow-up from primary clinicians. Diagnoses of CIDP, GBS and multiple sclerosis were made based on published criteria (McDonald *et al.*, 2001; Van den Bergh *et al.*, 2010; Wakerley *et al.*, 2014). Written informed consent was obtained from each individual. This study was approved by the Ethics Committee of Dokkyo Medical University and National University of Singapore.

Nerve and dorsal root ganglion staining

Teased fibres from sciatic nerves and dorsal root ganglion (DRG) sections of adult C57BL/6J mice were prepared as previously described (Lonigro and Devaux, 2009). Teased fibres were immersed in -20°C acetone for 10 min, blocked for 1 h in blocking solution containing 5% fish skin gelatin, 0.1% TritonTM X-100 in phosphate-buffered saline and incubated overnight at 4°C with sera diluted at 1:200 and mouse antibodies against voltage-gated sodium channels (1:500; Sigma-Aldrich) or goat antiserum against CNTN1 (1:2000; R&D systems). The slides were washed and incubated with the appropriate Alexa-conjugated secondary antibodies (1:500; Jackson ImmunoResearch). Slides were mounted and examined using an ApoTome fluorescence microscope (Carl Zeiss MicroImaging).

Cell-binding assay

Human embryonic kidney cells were plated onto poly-L-lysine coated glass coverslips in 24-well plates at a density of 50 000 cells/wells and were transiently transfected with CNTN1 constructs (Supplementary material) using JetPEI (Polyplus-transfection). The day after, cells were incubated with serum-free Opti-MEM[®] medium (Life technologies) for 24 h. Living cells were incubated for 20 min with serum diluted at 1:200 in Opti-MEM[®] with Alexa 594-conjugated anti-human IgG antibodies (1:500). After several washes, cells were fixed, permeabilized, and incubated for 1 h with mouse monoclonal antibodies against Myc (1:500; Roche) or a goat antiserum against CNTN1 (1:2000). Coverslips were revealed with secondary antibodies, and mounted. In some experiments, cells were transiently transfected with CNTN1 for 4 h, then treated with tunicamycin (2 $\mu\text{g}/\text{ml}$; Sigma-Aldrich) for 16 h prior to fixation and immunostaining. Neuron-binding assay, deglycosylation of CNTN1, immunoprecipitation and mass spectrometry are described in the Supplementary material.

Enzyme-linked immunosorbent assay

Human recombinant CNTN1 and contactin 2 (CNTN2) proteins were purchased from Sino Biological Inc. IgG, IgA and IgM antibodies against CNTN1 and CNTN2 were tested as described elsewhere (Miura *et al.*, 2014). Serum was considered positive when the calculated optical density was ≥ 0.1 at 1:500 dilution (Supplementary material). Each sample was tested in triplicate. Subclass of anti-CNTN1 IgG antibodies is described in the Supplementary material. A complement deposition assay using CNTN1 or GM1 as antigens was performed as described previously (Sudo *et al.*, 2014).

Statistics

Statistical analysis was performed by StatView version 5.0 (SAS Institute). *P*-values < 0.05 were considered as significant.

Results

Identification of CNTN1 as a target for autoantibodies in CIDP

To determine the antibody targets, we examined a patient with CIDP (Patient 1 in Table 1) presenting a strong IgG binding at paranodes and for whom the target antigen was unknown (Fig. 1A). The patient's IgG reacted with a surface antigen expressed in neocortical neurons (Fig. 1B). To identify the antigen, we immunoprecipitated proteins from neocortical neurons with the patient's serum. The serum pulled down a protein doublet of nearly 140 kDa (Fig. 1C). This doublet was identified by mass spectrometry as CNTN1.

Association of CIDP with anti-CNTN1 IgG4 antibodies

These results prompted us to screen a large cohort of patients with CIDP and GBS. IgG autoantibodies against CNTN1 were identified in 16 sera from patients with CIDP and five with GBS (Table 2), but not in multiple sclerosis patients and normal subjects. No IgG antibodies against CNTN2 were detected, and neither IgM nor IgA antibodies to CNTN1 were found. IgG4 antibodies to CNTN1 were identified in 13 of 16 patients with CIDP, but none of those with GBS. IgG2 antibodies to CNTN1 were identified in three patients with CIDP and in the five patients with GBS. In parallel, we blindly tested the sera on mouse teased fibres. Of interest, all the IgG4-positive CIDP sera strongly reacted against the paranodal domains (Patients 2 and 9 are shown in Fig. 1D and F) but not the IgG2-positive CIDP or GBS sera. This showed the association between the nerve staining and anti-CNTN1 IgG4 antibodies (Fisher's exact test, $P < 0.001$), suggesting that only the IgG4 antibodies are pathogenic. The presence of anti-CNTN1 IgG4 antibodies was significantly more frequent in CIDP than GBS, multiple sclerosis and normal controls (Fisher's exact test, $P = 0.02$).

We then tested whether these sera activate the complement pathway *in vitro*. None of the sera with anti-CNTN1 IgG4 antibodies activated the complement pathway or induced the deposition of the immune complex on ELISA plates. As controls, sera from two patients with GBS, which presented anti-GM1 IgG antibodies, induced the deposition of activated C3 components on ELISA plates. These results suggest that anti-CNTN1 IgG antibodies do not fix complement.

Clinical features of anti-CNTN1 IgG4-positive CIDP

Table 1 shows the clinical features of the anti-CNTN1 positive patients with CIDP. All 13 patients showed areflexia or hyporeflexia. To compare the clinical features, 50

anti-CNTN1 negative patients with CIDP were randomly chosen using a computer program. Age, sex and severity showed no differences between the two groups (Supplementary Table 1). Three of the 13 (23%) anti-CNTN1-positive patients with CIDP showed subacute symptom onset, whereas only one of the 50 (2%) anti-CNTN1-negative patients did (Fisher's exact test, $P = 0.04$). All of the anti-CNTN1 positive patients presented with sensory ataxia, whereas only 10 of the negative patients did (χ^2 test, $P = 0.02$). Only 4 of 10 (40%) anti-CNTN1-positive patients had a good response to intravenous immunoglobulin compared to 25 of 36 (69%) anti-CNTN1-negative patients (Fisher's exact test, $P = 0.18$). In contrast, 8 of 11 (73%) anti-CNTN1-positive patients had good responses to corticosteroids, whereas only 14 of 29 (48%) anti-CNTN1 negative patients did (χ^2 test, $P = 0.3$). Taken together, these results indicate that anti-CNTN1 IgG4 antibodies are associated with CIDP patients showing sensory ataxia and a tendency towards a good response to corticosteroids.

Expression of CNTN1 in large DRG neurons

Because patients with anti-CNTN1 IgG4 antibodies showed sensory ataxia, we investigated the localization of CNTN1 in DRG neurons. We found that CNTN1 is widely expressed in large DRG neurons (Fig. 1E), but not in small nociceptive neurons stained with voltage-gated sodium channel antibodies (Rasband *et al.*, 2001). Similarly, CIDP sera with anti-CNTN1 IgG4 antibodies stained large neurons in DRG sections and co-localized with CNTN1 staining in the soma and at the paranodes of sensory axons (Patient 9 in Fig. 1F). These results confirmed that anti-CNTN1 antibodies can target large diameter sensory neurons and axons.

Recognition of CNTN1 protein core by autoantibodies

To determine the targeted epitopes, we truncated the six immunoglobulin (Ig) domains or the four fibronectin type III (Fn) domains of CNTN1. Ten of 13 anti-CNTN1 IgG4-positive sera reacted with CNTN1 on human embryonic kidney cells (Patients 1 to 10). All these sera recognized the Ig domains, but not the Fn domains (Fig. 2A–F). Notably, eight sera bound to the Ig domain 5–6 (Patients 1 to 8). Because the Ig domains contain multiple potential N-glycosylation sites, but no O-glycosylation sites, we tested whether tunicamycin treatment could block antibody recognition. As non-glycosylated CNTN1 is retained in the endoplasmic reticulum, cells were fixed and permeabilized prior to staining. The reactive sera recognized CNTN1 even after tunicamycin treatment, suggesting that antibodies recognize the protein core.

Table 1 Clinical and laboratory features of chronic inflammatory demyelinating polyneuropathy patients with anti-contactin 1 IgG4 antibodies

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Diagnosis	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite	Typical / definite
Age / sex	75 / M	81 / M	63 / M	58 / M	33 / F	71 / M	59 / F	70 / M	47 / F	60 / M	63 / M	72 / M	36 / M
Modified Rankin scale at diagnosis	4	4	3	3	4	5	3	5	3	4	4	4	5
Initial symptoms	Numbness in both legs	Distal numbness	Numbness in both legs	Distal numbness	Distal numbness	Left hand weakness	Distal numbness	Gait disturbance	Distal numbness	Distal numbness	Distal numbness	Numbness in both legs	Distal numbness, gait disturbance
Clinical manifestations													
Onset	Chronic	Chronic	Chronic	Subacute	Chronic	Subacute	Chronic	Chronic	Subacute	Chronic	Chronic	Chronic	Chronic
Limb weakness	progressive Leg dominant, moderate	progressive Leg dominant, moderate	progressive Leg dominant, mild	progressive Leg dominant, mild	progressive Leg dominant, mild	progressive Leg dominant, severe	progressive Diffuse, mild	progressive Diffuse, severe	progressive Distal dominant, mild	progressive Diffuse, mild	progressive Diffuse, mild	progressive Distal dominant, mild	progressive Diffuse, severe
Sensory disturbance	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Deep sensation, distal dominant, severe	Distal dominant, severe
Other	Ataxia	Ataxia	Ataxia, tremor	Ataxia (truncal)	Ataxia	Ataxia, dysautonomia	Ataxia (truncal)	Ataxia, stupor, myasthenia	Ataxia, dysgeusia	Ataxia, dysgeusia	Ataxia	Ataxia, dysphagia	Ataxia, stupor, tremor, pseudoathetosis, facial and oropharyngeal weakness, dysgeusia
Cerebrospinal fluid findings													
Cell count (cell/mm ³)	4	10	Not available	1	6	6	4	6	Not available	2	2	2	0
Protein (mg/dl)	261	169	380	79	102	693	182	385	150	192	280	185	159
MRI abnormality	Not done	Normal	Not done	Not available	Normal	Normal	Nerve root hypertrophy	Not done	Not done	Normal	Normal	Normal	Brachial plexus swelling
Electrophysiological findings													
Prolonged motor distal latency	+	+	Not available	Not available	+	+	+	+	+	+	+	+	+
Reduction of MCV	+	+	Not available	Not available	No	+	+	+	+	Normal	+	+	+
Prolonged F-wave latency	+	Not evoked	Not available	+	+	Not available	+	Not done	Not available	+	Not done	Not evoked	Not done, because of marked decreased distal CMAPs
Conduction block	Not available	+	Not available	Not available	No	+	+	No	No	+	No	+	No
Excessive temporal dispersion	Not available	+	Not available	Not available	No	+	+	+	+	+	No	+	No
Sural nerve biopsy	Not done	No demyelination	Not done	Not done	Not done	Axonopathy	Not done	Axonal degeneration and paranodal demyelination	Poor study	Not done	Not done	Not done	Not done
Treatment													
Intravenous immunoglobulin	Ineffective	Partially effective	Not done	Partially effective	Not done	Ineffective	Ineffective	Ineffective	Not done	Effective	Ineffective	Partially effective	Ineffective
Corticosteroids	Effective	Partially effective	Effective	Not done	Effective (remission)	Not done	Effective (remission)	Ineffective	Effective	Effective	Ineffective	Partially effective	Ineffective
Other	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Cyclophosphamide pulse; ineffective	Not done	PE, effective	Cyclophosphamide pulse; ineffective PE; partially effective	Not done	Tacrolimus and cyclosporin; ineffective PE; ineffective

CMAP = compound muscle action potential; MCV = motor nerve conduction velocity; PE = plasma exchange.

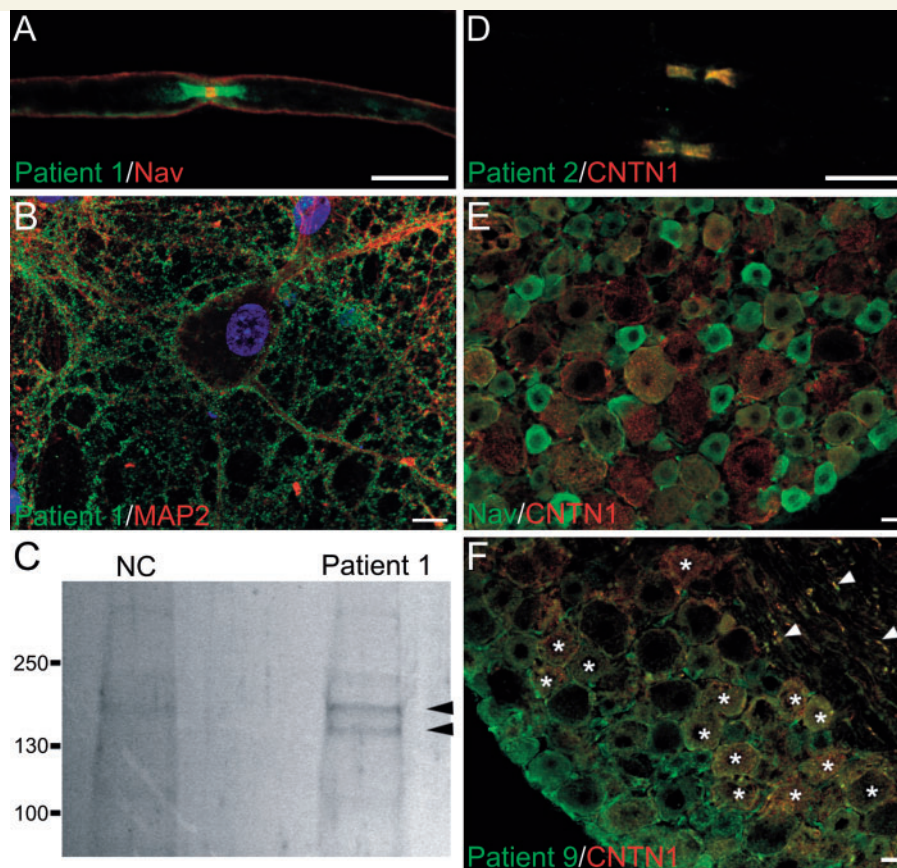


Figure 1 Identification of CNTN1 as a target for autoantibodies in CIDP. (A) The sera from a CIDP patient (Patient 1) was tested on mouse sciatic nerve fibres. Human IgG antibodies (green) bound specifically to the paranodal regions, which flank voltage-gated sodium (Nav) channels (red) at nodes. (B) The IgG (green) from the same CIDP patient labelled the surface of cultured neocortical neurons, here stained with microtubule-associated protein 2 (MAP2) (red). Scale bar = 10 μ m. (C) Neocortical neurons were incubated with normal control (NC) (left) and CIDP (right) IgG antibodies, and the target antigens were immunoprecipitated, separated on SDS-PAGE gels, and stained with Imperial blue. Protein bands around 140 kDa (arrowheads) were excized and identified by mass spectrometry as CNTN1. Molecular weight markers are shown on the left in kDa. (D) The CNTN1 reactive sera were then tested by immunostaining on mouse teased nerve fibres. All the anti-CNTN1 IgG4 antibody-positive patients showed a clear IgG binding (green) at paranodal regions, which co-localized with CNTN1 (red). Here we show immunolabelling obtained with a CIDP patient (Patient 2). Scale bar = 10 μ m. (E and F) Dorsal root ganglion sections were immunostained for CNTN1 (red) and Na⁺ channels (green; E) or a representative CIDP serum (green; Patient 9; F). CNTN1 was found in large DRG neurons, whereas Na⁺ channel staining was more prominent in small neurons. CIDP IgG antibodies bound preferentially to large CNTN1-positive neurons (asterisks) and co-localized with CNTN1 at paranodes of sensory axons (arrowheads). Scale bars = 20 μ m.

To confirm these results, we performed deglycosylation experiments using peptide *N*-glycosidase F, which cleaves *N*-linked glycans. Untreated CNTN1 appeared as a protein doublet around 140 kDa, reflective of the two glycosylated forms of CNTN1 (Fig. 2G). However, after the peptide *N*-glycosidase F treatment, CNTN1 appeared as a single band ~100 kDa. Of interest, CIDP sera recognized both the glycosylated and deglycosylated forms of CNTN1.

Discussion

In our previous study, we found anti-CNTN1 IgG antibodies in 1 of 50 (2%) patients with CIDP (Devaux *et al.*, 2012). Interestingly, in the previous study, IgG antibodies

from Patient 1 did not react against rat CNTN1 by cell-binding assay. However, in the current study we identified CNTN1 as a target for the IgG autoantibodies using an unbiased proteomic approach. We also showed that the IgG4 antibodies specifically recognize the paranodes on teased nerve fibres and human CNTN1 by ELISA. These results suggested that cell-binding assay alone may not be sensitive enough as a screening method, and that we may have underestimated the prevalence of anti-CNTN1 antibodies.

In this retrospective study, anti-CNTN1 IgG4 antibodies were detected in 13 of 533 (2.4%) patients with CIDP and were significantly associated with CIDP. Our data thus support a previous report where anti-CNTN1 IgG4 were identified in 3 of 46 (7%) Spanish patients with CIDP

Table 2 Association of paranodal staining with anti-contactin I IgG4 antibodies

Diagnosis	Patient No.	IgG titres	IgG subclass titres				Paranodal staining
			IgG1	IgG2	IgG3	IgG4	
CIDP	1	32 000	8000	2000	1000	16 000	+
	2	128 000	16 000	8000	2000	128 000	+
	3	16 000	4000	2000	1000	16 000	+
	4	16 000	2000	2000	0	16 000	+
	5	32 000	8000	16 000	1000	128 000	+
	6	64 000	16 000	4000	2000	128 000	+
	7	64 000	32 000	2000	1000	32 000	+
	8	64 000	8000	0	0	16 000	+
	9	16 000	2000	0	0	16 000	+
	10	8000	0	0	0	4000	+
	11	32 000	4000	0	0	32 000	+
	12	32 000	4000	0	0	32 000	+
	13	1000	0	0	0	1000	+
	14	32 000	1000	64 000	0	0	—
	15	4000	1000	1000	0	0	—
	16	1000	2000	4000	0	0	—
GBS	17	4000	1000	2000	0	0	—
	18	2000	1000	1000	0	0	—
	19	4000	1000	8000	0	0	—
	20	4000	0	16 000	0	0	—
	21	1000	0	500	0	0	—

(Querol *et al.*, 2013). In the latter study, the authors highlighted aggressive symptom onset in their patients. Albeit in a preliminary work, we did not detect anti-CNTN1 IgG antibodies in 14 patients with acute-onset CIDP (Miura *et al.*, 2014), here we found anti-CNTN1 IgG4 antibodies in a subset of patients with acute-onset CIDP within a large cohort of patients with CIDP. This supports that CIDP and acute-onset CIDP form a continuous spectrum. In addition, anti-CNTN1 IgG4 antibodies appear as a potent biomarker to differentiate acute-onset CIDP from GBS, and may thus help to choose appropriate immunotherapy for each condition.

In keeping with this view, we found that patients with CIDP with anti-CNTN1 IgG4 antibodies presented a poor response to intravenous immunoglobulin, thus confirming a previous observation (Querol *et al.*, 2013). However, two-thirds of our patients positively responded to corticosteroids, highlighting that these autoantibodies could serve as a biomarker to guide treatment option. Antibody assays to detect anti-CNTN1 IgG4 antibodies can be easily performed, assisting clinicians to select the best treatment. Nerve staining can provide useful complementary information to confirm positive results.

Here we noted that all of the patients with anti-CNTN1 IgG4 antibodies presented with sensory ataxia. The predominant sensory symptoms were in keeping with the finding that CNTN1 is strongly expressed in large DRG neurons. Therefore, it is plausible that anti-CNTN1 antibodies preferentially affect sensory axon paranodes. In keeping with this, anti-CNTN1 autoantibodies have been

found to alter paranodal junctions in myelinating co-cultures of Schwann cells/DRG (Labasque *et al.*, 2014). IgG4 does not bind Fc receptors and does not activate the complement pathway (Nirula *et al.*, 2011). In accordance, IgG antibodies from our patients did not activate complement. These antibodies may thus have an antigen-blocking function and may block the interaction between CNTN1 and its partners CNTNAP1/Caspr1 and neurofascin-155 at paranodes, as was suggested *in vitro* using cell aggregation assays (Labasque *et al.*, 2014).

Nonetheless, the clinical features of our patients contrasted with a previous study where only one of four patients showed ataxia (Querol *et al.*, 2013). The reasons for this discrepancy are unclear. We first suspected that the autoantibodies might target different epitopes. We found that IgG4 antibodies reacted against the Ig domains of human CNTN1 and recognized the glycosylated and unglycosylated proteins. In addition, using an unbiased biochemical assay, we demonstrated that antibodies from patients with CIDP recognized the two glycosylated forms of CNTN1 (mannose-rich N-glycans and complex N-glycans) and the core protein of deglycosylated CNTN1. By contrast, IgG4 antibodies from the four Spanish patients with CIDP recognized N-glycans within the Ig domains of rat CNTN1 (Labasque *et al.*, 2014). This strongly supports the hypothesis that IgG4 antibodies from these patients recognize different epitopes, and thus target different axonal populations and induce different clinical symptoms. However, we cannot exclude that these authors have overlooked antibody binding to unglycosylated CNTN1.

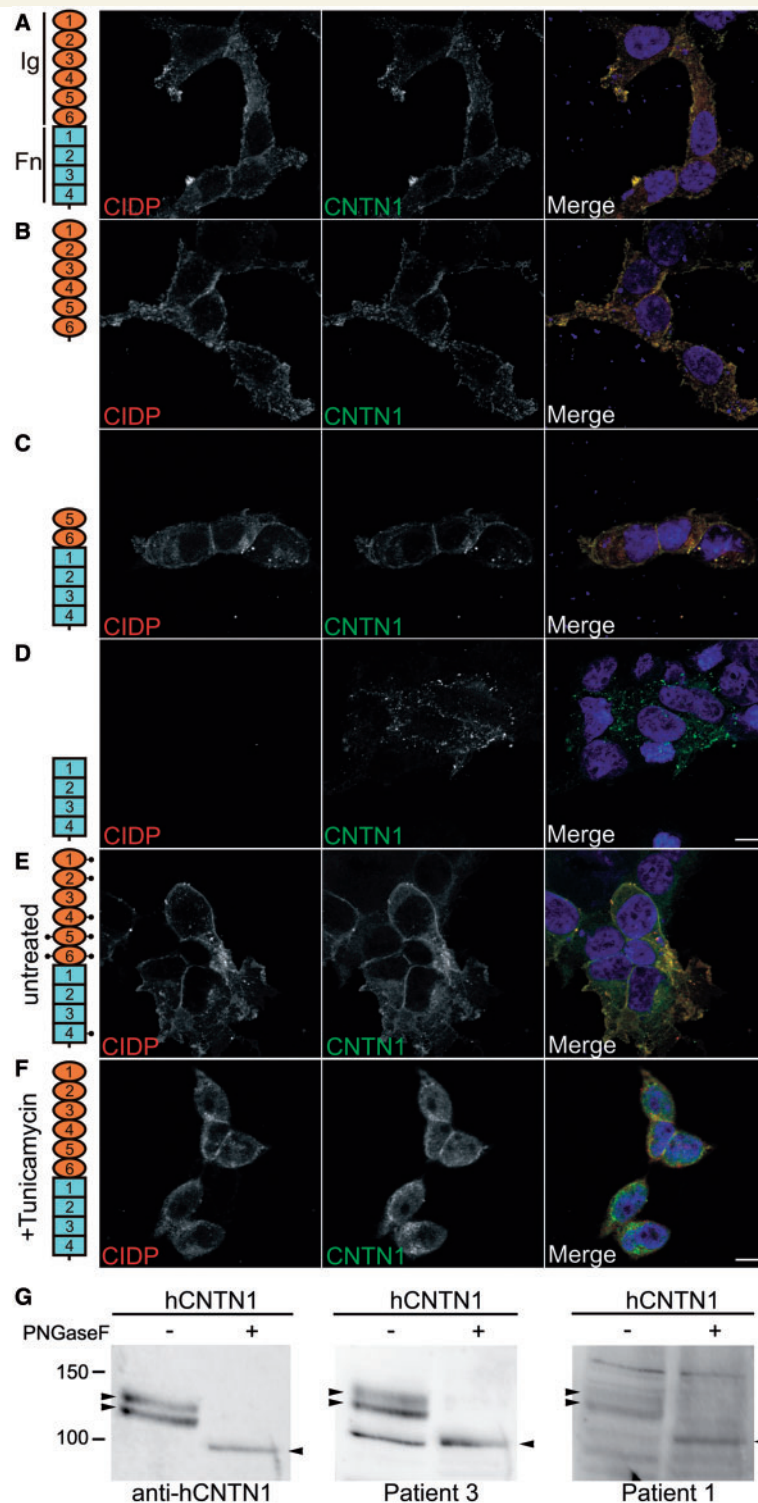


Figure 2 CIDP autoantibodies recognize the protein core of CNTN1 and are directed against the Ig domains. (A–D) Human embryonic kidney cells were transfected with constructs encoding full-length CNTN1 (A), Ig domains 1–6 (B), Ig domains 5–6 + fibronectin type III (Fn) domains (C), or Fn domains (D). Living cells were then incubated with a representative CIDP patient's serum (red), fixed, and stained for CNTN1 (green). CIDP IgG antibodies recognized the Ig domains of CNTN1, but did not bind the Fn domains. (E and F) Human embryonic kidney cells were transfected with full-length CNTN1, then treated with tunicamycin (F) or normal medium (E). Cells were then fixed, permeabilized and stained for CNTN1 (green) and CIDP IgG (red). Serum IgG antibodies recognized CNTN1 from tunicamycin-treated cells, indicating that the antibodies target the unglycosylated protein core. Scale bar = 10 μ m. (G) Protein samples from human CNTN1 (hCNTN1) transfected human embryonic kidney cells were untreated (–) or treated (+) with peptide N-glycosidase F (PNGaseF), and immunoblotted against CNTN1 (left) or two representative CIDP sera (Patients 3 and 1). The goat anti-CNTN1 antibodies recognized the two glycosylated forms of CNTN1 around 140 kDa (arrowheads on the left) and the deglycosylated protein core (arrowheads on the right). Similarly, CIDP IgG antibodies recognized both glycosylated and deglycosylated CNTN1. Molecular weight markers are shown on the left in kDa.

Indeed, we found that patients with CIDP react more potently against human CNTN1 compared to rat CNTN1 used in their study. In addition, tunicamycin treatment and point mutations can strongly impair protein stability, and preclude the detection of IgG antibody binding.

In conclusion, anti-CNTN1 IgG4 antibodies are associated with subacute onset of symptoms, sensory ataxia and good response to corticosteroids, being a possible biomarker to choose better immunotherapy. These results should motivate international study groups to investigate the frequency of the anti-CNTN1 IgG4 antibodies, clinical features and treatment responses of patients with CIDP among different countries.

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Conflicts of interest

Prof. Yuki serves as an editorial board member of *Expert Review of Neurotherapeutics*, *The Journal of the Neurological Sciences*, *The Journal of Peripheral Nervous System* and *Journal of Neurology, Neurosurgery & Psychiatry*.

Supplementary material

Supplementary material is available at *Brain* online.

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Appendix I

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