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Peripherin-IgG Association with Neurologic and Endocrine Autoimmunity

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Abstract

Peripherin-IgG has been reported a pertinent autoantibody in non-obese type 1 diabetic (NOD) mice. However, it has not previously been recognized in any human disease. In blinded evaluation of serum for markers of neurological autoimmunity in a high-volume diagnostic laboratory, we incidentally identified 26 patients (61% female) with an IgG that bound selectively to neural elements in enteric ganglia, sympathetic nerve trunks and discrete nerve tracts in mid-brain and hind-brain. The target antigen was identified as peripherin, a 55 kDa-type III intermediate filament protein. Review of clinical histories revealed that 54% of seropositive patients had dysautonomia (predominantly gastrointestinal dysmotility), 30% had neuropathies with varied sensory symptoms and 35% had clinical or serological evidence of endocrinopathy (type 1 diabetes, thyroiditis or premature ovarian failure). Collectively, 73% had autonomic dysfunction or endocrinopathy. None of 173 healthy subjects was seropositive. Subsequent western blot evaluation of archival sera from patients with small fiber/autonomic neuropathies (with or without endocrinopathy) revealed a 33% seropositivity rate for peripherin IgG. Our further demonstration that peripherin-immunoreactive autonomic fibers in pancreas, thyroid and ovary are juxtaposed to endocrine epithelium, complement our clinical observations in suggesting that neuronal elements may be a pertinent initial target for immune attack in multiple forms of endocrine autoimmunity (intermolecular epitope spreading). It remains to be determined whether or not peripherin-IgG is predictive for development of small fiber neuropathy (autonomic or somatic).

Keywords

Autoimmune endocrinopathy; dysautonomia; peripheral neuropathy; NOD mice

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1. Introduction

Neural-restricted autoantibodies serve as valuable serum biomarkers aiding the diagnosis of acquired neurological disorders amenable to immunotherapy, both idiopathic and paraneoplastic. Informative autoantigens defined to date are expressed in neurons, glia or skeletal muscle. Plasma membrane-reactive IgGs have pathogenic potential [1-6], while IgGs reactive with cytoplasmic antigens [7-11] are indicative of immunogenic proteins yielding peptide targets for MHC-restricted cytotoxic T cells [12]. In our clinical service laboratory's high volume experience of screening patients' sera by immunofluorescence for markers of neurological autoimmunity, we identified incidentally a novel IgG that bound selectively to peripheral autonomic neurons and to discrete nerve fiber tracts in the central nervous system. Review of records for initially identified seropositive Mayo Clinic patients suggested that this IgG was a marker of autoimmune dysautonomia. Here we describe the clinical profiles of 26 seropositive patients and define the neuronal intermediate filament protein peripherin as the target autoantigen. Peripherin-IgG has been reported a pertinent autoantibody in non-obese type 1 diabetic (NOD) mice, but it has not previously been recognized in any human disease. We propose a novel hypothesis that plausibly unifies our clinical and immunohistochemical observations.

2. Patients and Methods

2.1 Patients and controls

The study was approved by the Mayo Clinic Institutional Review Board (IRB #06-007020). Between January 1, 1998 and December 31, 2008, the clinical Neuroimmunology Laboratory prospectively screened on a service basis sera from approximately 160,000 patients for evidence of neurological autoimmunity using an indirect immunofluorescence assay to detect IgG binding selectively to neural elements in a composite substrate of mouse tissues [9]. An occasionally detected IgG bound to discrete filamentous elements in the central and peripheral nervous system. Review of clinical correlations in the first 5 identified patients suggested a significant association with autonomic dysfunction, particularly gastrointestinal dysmotility. To define the novel autoantibody's clinical accompaniments more rigorously, we prospectively collected an additional 21 patients whose sera yielded this immunostaining pattern and for whom adequate clinical information was available. Two neurologist coauthors (MA and SJP) reviewed records of seropositive patients, and extracted and tabulated pertinent history, physical examination findings and laboratory reports (imaging, electrophysiological, physiological [autonomic reflex screen and thermoregulatory sweat test] and autoimmune serology). We tested additionally 173 age-matched healthy control subjects and archival sera from 49 patients with clinical diagnoses of endocrine disorders and neuropathies.

2.2 Immunofluorescence assays

For clinical screening, patients' sera were diluted (1:240) in PBS containing 1% BSA, pre-absorbed with beef liver powder and applied to a composite substrate of 3 frozen tissues (adult mouse cerebellum/midbrain, gut and kidney, 4-μm-thick and post-fixed with 10% formalin). Sera yielding positive results were titrated in doubling dilutions to determine endpoints of antibody detection. For research analyses we used cultured cell substrates, attached to coverslips, washed in PBS, fixed in 95% ethanol/5% acetic acid (-20°C, 15 minutes), rinsed in PBS, and permeabilized by 3 minutes' exposure to 0.05% Triton ×100. All substrates were blocked with normal goat serum (10% in PBS; 15 minutes). Commercial antibody probes were diluted in block buffer. After applying primary antibodies (45 minutes), and washing, we applied species-appropriate secondary antibodies (45 minutes), rinsed the substrates, applied ProLong anti-fade medium (Molecular Probes P36935) and mounted coverslips. Multiple antigen labeling was performed on mouse tissues (female, aged 6-8 weeks), oriented in OCT

and snap frozen in isopentane. Cryosections (8 μm) were air-dried, incubated sequentially in PBS containing 10% normal goat serum or 1% BSA (30 minutes), primary antibody (45 minutes) and secondary antibody (45 minutes). After washing, the sections were mounted in ProLong anti-fade agent containing DAPI and analyzed by confocal microscopy. Animal studies were approved by the Mayo Institutional Animal Use and Care Committee (A36207).

2.3 Tests for other organ-specific autoantibodies

We used radioimmunoprecipitation assays to detect cation channel antibodies specific for muscle and ganglionic neuronal ACh receptors, voltage-gated neuronal channels for calcium (P/Q-type and N-type) and potassium (α-dendrotoxin sensitive channels), and GAD65, IA-2 and insulin antibodies (using ^{125}I-labelled recombinant human antigens [Kronus, Inc.]). We detected skeletal muscle striational Abs by ELISA [13], CRMP-5-IgG by western blot using recombinant human protein, and thyroid peroxidase and thyroglobulin antibodies by agglutination assays [9].

2.4 Rat cell lines

Cells were plated on poly-L-lysine-coated glass coverslips (BD BioCoat 354085) and held at 37°C in a humidified atmosphere of 5-7% CO_2/95-93% air. Culture media were supplemented with L-glutamine, 1 mM (Sigma-Aldrich G8540-100G) and penicillin/streptomycin (Invitrogen 15140-163). PC12 pheochromocytoma cells (ATCC CRL-1721) were maintained in DMEM 4.5 (Invitrogen 12100-061) supplemented with 10% fetal calf serum (Atlas Biologicals, Cat No. F-0500-A), 10% horse serum (HyClone Labs, Cat No. A-3311-L). To promote differentiation, mouse nerve growth factor (2.5S; Alamone Laboratory, Cat No. N-100) was added on alternate days for 7 days (100 ng/mL). L6 skeletal muscle cells (ATCC, CRL-1458) were maintained in DMEM 4.5 supplemented with 10% horse serum. To promote differentiation, 2% fetal calf serum was substituted for 3 days; myotube formation was confirmed by microscopic examination. CG4 oligodendroglial-astrocytic progenitor cells, provided by Dr. Charles Howe, Department of Neurology, Mayo Clinic, Rochester, MN, were grown in proliferation medium and differentiated as described previously [3].

2.5 Antibody probes

Rabbit anti-neurofilament M (Chemicon AB1987), Cy3-conjugated chicken anti-GFAP IgG (Sigma C9205), rabbit anti-peripherin (Chemicon AB1530), mouse anti-peripherin (Chemicon MAB1527), goat anti-PDX-1 (Abcam ab47383) and human serum containing IgG reactive with skeletal muscle contractile proteins (myasthenia gravis patient, 83-4868). Species-specific anti-IgG antibodies, conjugated with fluorochrome or horseradish peroxidase, were from Southern Biotechnology Associates, Inc.

2.6 Protein extraction and western blotting

PC12 cells (100 μL packed volume) were extracted in 2 mL buffer (0.15 M NaCl, 0.01 M $NaPO_4$, 2 mM EDTA, pH 7.2) containing 1% NP40, 0.1% SDS and protease inhibitors (Complete™, Roche 11697498001). After shaking (4°C, 1 hour), proteins were denatured and reduced by boiling (5 minutes) in 2% SDS and 2-mercaptoethanol, separated by electrophoresis in 10% polyacrylamide gel, and transferred to nitrocellulose for western blot. Molecular weight standards included biotinylated broad range markers (BioRad 161-0322) or pre-stained SDS-PAGE standards (BioRad 161-0374).

2.7 Affinity purification of patient IgG

PC12 lysate proteins were transblotted to nitrocellulose. Bound antigenic protein was located by western blot staining of excised vertical edge strips. The horizontal intervening strip bearing antigen, and a control horizontal strip lacking the antigen of interest, were probed with patient

serum. Both were washed thrice in high salt buffer (50 mM Tris-HCl, pH 7.4 containing 500 mM NaCl), and once in low salt buffer (100 mM NaCl). Bound IgG was eluted in acidic glycine, neutralized, dialyzed against PBS, 0.02% sodium azide and applied to the composite mouse tissue substrate to evaluate the immunofluorescence staining pattern.

2.8 Sub-cellular fractionation

Four fractions (cytosol, membrane, nucleus and cytoskeleton) were prepared from PC12 cells using a Subcellular Proteome Extraction kit (Calbiochem 539790). After electrophoresis in denaturing 10% polyacrylamide gel, proteins were transblotted to nitrocellulose and probed with positive patient serum.

2.9 Two-dimensional (2D) electrophoresis, in-gel trypsin digestion and mass spectrometry

The fraction containing the putative antigen was separated by 2D electrophoresis using published methods [14]. Proteins were visualized by silver staining and immunoblotting. Prominent antigenic spots were excised and subjected to in-gel digestion and analysis by tandem mass spectrometry [15]. Peptides were identified using the MASCOT search algorithm [16].

3. Results

3.1 Clinical-serological correlations of a novel neural autoantibody

We identified a total of 26 seropositive patients for whom clinical records were available (16 women, 10 men). We did not detect the novel autoantibody in any of 173 age-matched healthy control subjects. Table 1 summarizes the 26 seropositive patients' clinical and laboratory information and serological data. Their median age was 46 years (range 21-86); the median titer of the novel IgG was 3,840 (range, 240 to 30,720). All but 8 patients were evaluated at Mayo Clinic. All had neurological complaints. Dysautonomia was the most commonly documented clinical association (14 patients; 54%). Nineteen patients (73%) had dysautonomia or endocrinopathy. Dysautonomia was generalized in 2 patients and limited in 12; 8 had gastrointestinal (GI) dysmotility and 6 had abnormalities of sudomotor, cardiovagal or adrenergic functions. GI dysmotility was confirmed by endoscopy, manometry or transit studies. Diverse neurological manifestations were documented in individual patients. Neuropathies with varied sensory symptoms were common. Other neurological diagnoses included sensorimotor neuropathy, lumbosacral plexopathy, transverse myelitis, non-specified myelopathy, optic neuropathy, encephalitis, cerebellar ataxia and myasthenia gravis. Three patients had a history of neoplasia. Six patients (23%) had one or more coexisting neural autoantibodies (neuronal voltage-gated calcium or potassium channels, GAD65, ganglionic acetylcholine receptor, CRMP-5), 4 had other endocrine autoantibodies and 5 had non-organ-specific autoantibodies (Table 2).

Nine patients (35%) had a clinically documented endocrinopathy, a marker autoantibody of endocrine autoimmunity or both. Endocrinologic disorders documented in 7 patients included insulin-dependent diabetes, 4 (juvenile and adult-onset, with coexisting endocrine disorders or autoantibodies, 3), thyroid disorders, 5, and premature menopause, 2. Two patients had thyroid autoantibodies (thyroperoxidase [2] or thyroglobulin [1]) without documented thyroid dysfunction (Table 2).

3.2 Immunohistochemical characteristics

After routine pre-absorption with liver powder, healthy control sera, and a majority of service-tested patients (thousands monthly), yield totally negative immunofluorescence staining of the mouse tissue substrate. Figure 1 illustrates the distinctive pattern of human IgG

immunostaining yielded by sera of this study's patients. In stomach (upper panel), immunoreactive elements were prominent in neural elements of smooth muscle (enteric ganglia and nerve trunks), mucosa and submucosa (nerve fibers). In kidney (middle panel), immunoreactivity was restricted to sympathetic nerve trunks and fibers near arteries and arterioles. The mid-hind brain (lower panel) contained discrete immunoreactive nerve tracts.

3.3 Neurons contain abundant autoantigen

To identify unambiguously the cell types expressing immunoreactivity, we investigated a panel of rat cell lines by staining with patient IgG and defined IgG probes reactive with cell-type appropriate filaments (Figure 2) in PC12 pheochromocytoma cells, CG4 glial cells and L6 skeletal muscle cells (respectively, neurofilaments, GFAP intermediate filaments and sarcomeric striational antigens [17]). The novel human autoantibody bound to filaments that were restricted to the cytoplasm of PC12 neuronal cells. We used PC12 thereafter as the source of autoantigen for immunochemical and molecular analyses.

Western blot defined an IgG in patient sera (18 of 18 tested) that bound to a PC12 lysate protein of Mr ~55 kDa (Figure 3a). To verify that this protein represented the antigen defined by immunostaining, we transferred electrophoretically-separated proteins to nitrocellulose and probed edge strips with patient IgG to identify the immunoreactive band. The horizontal strip containing immunoreactivity, plus an irrelevant strip containing lower molecular weight proteins, were excised and exposed to patient serum or control human serum. After washing extensively, we eluted bound IgGs and applied them to the mouse triple tissue substrate. IgG eluted from the ~55kDa protein strip, but not from the irrelevant protein strip, yielded the characteristic staining pattern observed with the patient's original serum (Figure 3b-d).

3.4 Identification of the neuronal autoantigen

Western blot analysis of PC12 lysate fractions enriched for cytosol, membrane, nucleus or cytoskeleton demonstrated that IgG in the patient sera bound exclusively to a cytoskeletal protein (Figure 3e). Initial attempts to purify the antigen were hampered by its insolubility. We succeeded by combining 2D electrophoresis, immunoblotting and mass spectrometry analyses. Silver staining and immunoblotting both revealed 4 identical spots (Figure 4). Analysis of spots 1-4 by in-gel digestion and mass spectrometry assigned 41, 47, 38 and 36 different peptides respectively to rat peripherin (accession no. P21807, Swiss-Prot data base).

3.5 Peripherin Is expressed in multiple endocrine tissues

We used rabbit anti-peripherin IgG, patient sera and adult mouse tissues to investigate the distribution of peripherin in the endocrine tissues for which 35% of the peripherin-IgG-positive patients in this report had clinical or serological evidence of autoimmunity, namely thyroid, pancreas and ovary. Brain served as a positive control tissue, and liver as a negative control tissue [18]. The autoantigen defined by patient IgG in brain co-localized with peripherin (Figure 5). Immunoreactivity was prominent in nerve fibers surrounding islets of Langerhans in the pancreas, in nerve fibers in interstitial tissue between thyroid follicles, and in nerve fibers adjacent to ovarian follicles. Immunoreactivity was not detected in liver.

3.6 Peripherin IgG associates with clinical neuropathies

To estimate the frequency of peripherin-IgG among patients in clinical diagnostic categories represented by the study patients in whom we incidentally detected seropositivity (Table 1), we tested by immunofluorescence and western blot (PC12 lysate) archival sera from three patient groups defined by clinical diagnosis (Table 3). None were positive by immunofluorescence. By western blot no patient was seropositive in groups with uncomplicated type 1 diabetes (n=28) or a combination of type 1 diabetes, premature

menopause or thyroiditis (n=9; most had a history of neoplasia). However, 4 patients of 12 (33%) in the group with small fiber neuropathy, with or without autonomic involvement, premature menopause or type 1 diabetes, were positive by western blot. Two were female and two were male (ages 16-57); 3 had both somatic and autonomic small fiber neuropathies. These preliminary data suggest that neuronal elements may be the initial target of autoimmunity (preceding endocrine autoimmunity). Longterm clinical and serological follow-up of large numbers of patients of this type would be required to test this hypothesis. For the present, however, peripherin-IgG appears to be a useful aid to diagnosing an autoimmune subset of patients with varied neurological presentations, most commonly autonomic/small fiber neuropathies.

4. Discussion

This is the first reported association of peripherin-specific IgG with human disease. Our study has defined peripherin-IgG as a clinically pertinent biomarker of autoimmune neuropathies (somatic and autonomic) often coexisting with endocrine autoimmunity. Peripherin-IgG was not found in any healthy control subject. Fifty-four percent of the 26 seropositive patients we identified had symptoms of limited or generalized dysautonomia, and 27% had a clinically diagnosed endocrinopathy. Collectively, 73% of the patients had autonomic dysfunction or clinical endocrinopathy affecting thyroid, pancreas or ovary. Other neurological manifestations involved the peripheral nervous system more than the central; 30% of seropositive patients had neuropathies with varied sensory symptoms. More than 99% of patients whose sera are referred to the Mayo Clinic Neuroimmunology Laboratory for diagnostic immunofluorescence screening have a neurological disorder (usually subacute). However, only a small minority has dysautonomia, and there is no endocrinologic bias. Neuronal autoantibodies rarely have a syndrome-specific neurological correlate (Pittock et al, 2004). It is therefore remarkable that 76% of seropositive patients had evidence of small fiber neuropathy (autonomic or sensory). The results of our preliminary blinded analysis of sera selected by clinical diagnosis support our conclusion that peripherin autoimmunity is associated with acquired neuropathies with autonomic and varied sensory symptoms and an accompanying endocrinopathy.

Serum autoantibody profiles recognized in the past 2 decades have enabled identification of autoimmune cases of GI dysmotility as a limited form of dysautonomia [2,5,19-22]. Peripherin-IgG complements an existing informative profile of autoantibodies associated with autoimmune GI dysmotility: anti-neuronal nuclear autoantibody, type 1 (ANNA-1, aka “anti-Hu”), collapsin response-mediator protein-5 (CRMP-5)-IgG, N-type voltage-gated calcium channel, voltage-gated potassium channel, ganglionic and muscle acetylcholine receptor, striational and glutamic acid decarboxylase-65 antibodies [5,20-23]. To date, only ganglionic acetylcholine receptor-specific IgG is proven to be pathogenic for the autonomic nervous system [2,24,25]. Evidence is lacking for *in vivo* pathogenicity of IgGs specific for intracellular autoantigens. However, these antibodies are recognized as surrogate markers for antigen-specific T cell activation [12]. It is plausible that peripherin-containing nerve fibers may be susceptible to attack by activated effector cytotoxic T cells specific for peripherin-derived peptides, in the context of appropriate MHC molecule upregulation.

Peripherin is a type III neuronal intermediate filament protein that forms networks, either alone or complexed with other neurofilament proteins [26]. It is attributed a role in neuron development and repair [27] and is distributed widely in the peripheral nervous system. In the central nervous system peripherin is restricted to regions that project to the periphery. Mice lacking peripherin appear surprisingly normal, apart from having reduced numbers of unmyelinated fibers in ventral roots [28].

Peripherin has been proposed a candidate autoantigen of type 1 diabetes, based on the detection in diabetic NOD mice of peripherin-IgG in serum and on the specificity of antibodies produced by B lymphocytes infiltrating the pancreas [29-31]. The seroprevalence of peripherin-IgG in NOD mice is reported to parallel diabetes progression [32]. It is therefore remarkable that no previous study has demonstrated peripherin-IgG as a pertinent autoantibody in human disease, either endocrine or neurologic. Type 1 diabetes is estimated to affect 7.8% of the U.S. population, but it was diagnosed in 15% of the patients in this study; an additional patient had documented hyperglycemia. In a series of papers describing the evolution of diabetes in NOD mice, Carrillo and colleagues proposed that nervous tissue-specific B cells are recruited to the region of pancreatic islets as an early event, preceding β-cell destruction. They hypothesized that the expression of peripherin in pancreatic neuronal elements is upregulated by low level inflammation [29-31]. Pancreatic islets are richly innervated by autonomic nerves [33]. Electron microscopy has demonstrated, in pancreatic tissues of NOD mice and humans, that a tight envelope of peri-islet Schwann cells converges at the neuro-insular complex with axons and sympathetic nerve fibers. These Schwann cells have been implicated as the initial target of T lymphocyte attack in pre-diabetes. In male NOD mice, which are relatively resistant to diabetes, the peri-islet Schwann cell barrier remains intact [34]. The juxtaposition of peripherin-positive autonomic fibers and epithelia in all of the endocrine organs that we identified as targets of autoimmunity in the peripherin-IgG positive patients in our study, suggests that neural elements may be an early target for immune attack in multiple forms of human endocrine autoimmunity, including type 1 diabetes, premature ovarian failure and thyroid disorders. It remains to be determined whether or not peripherin-IgG is predictive for development of small fiber neuropathy (autonomic or somatic).

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Abbreviations used

CRMP	collapsin response-mediator protein
GI	gastrointestinal
GFAP	glial fibrillary acid protein
IF	immunofluorescence
NOD	non-obese diabetic mice

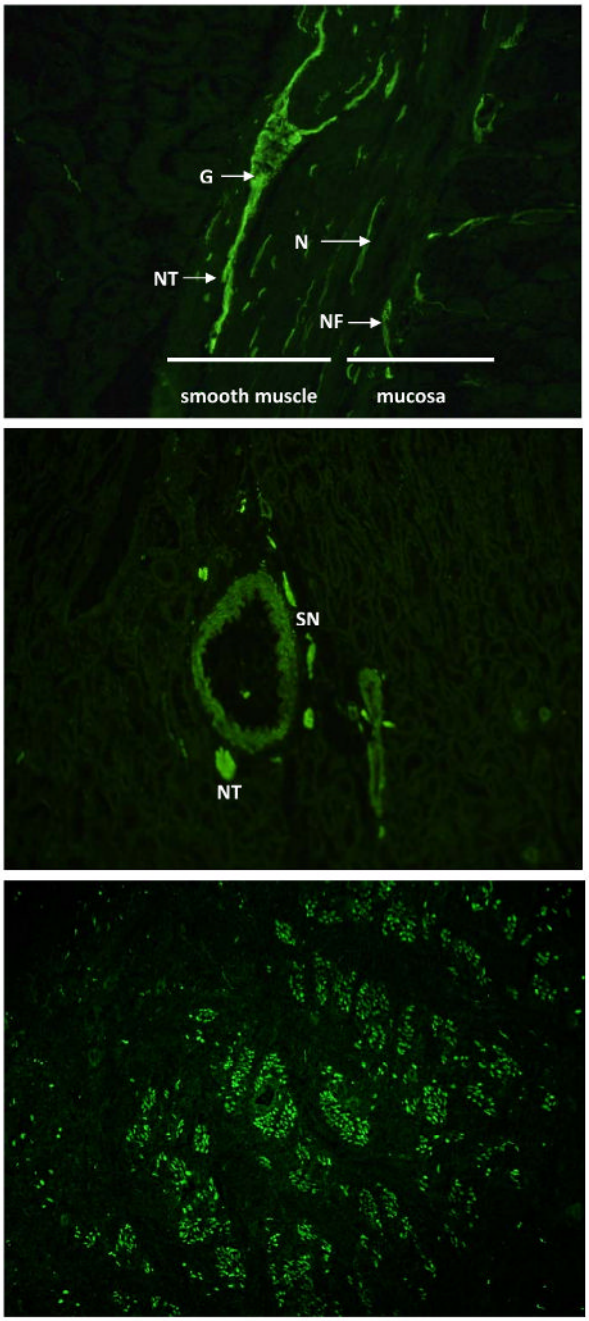

Fig. 1.

Novel IgG autoantibody binds to neural elements in sections of mouse stomach and kidney and to discrete fiber tracts in mid-hind brain. Bound IgG was visualized using fluorescein-conjugated anti-human IgG. The characteristic staining pattern of this autoantibody is prominent in myenteric ganglia, nerve fibers and nerve trunks in the enteric nervous system (upper panel), sympathetic nerve trunks and fibers adjacent to arterioles in the gastric submucosa and kidney (center panel) and discrete nerve bundles in the mid-hind brain (lower panel). G: ganglion; NT: nerve trunk; NF: nerve fibers; SN: sympathetic nerve

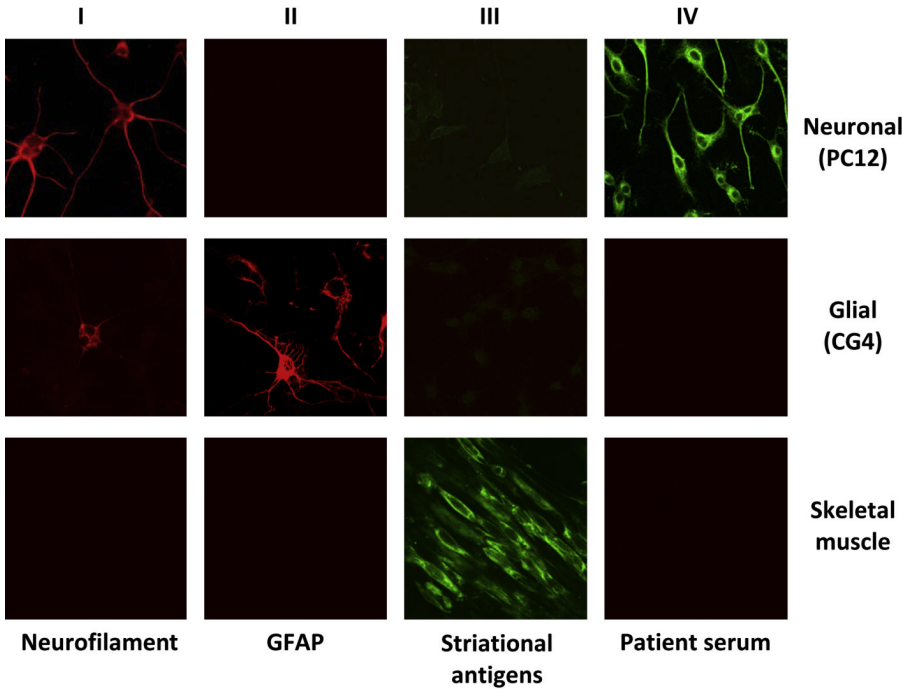

Fig. 2.

The autoantigen is restricted to neurons. Differentiated cell lines used as substrates: pheochromocytoma (PC12; upper panels), astrocytes (CG4; middle panels) and skeletal muscle (L6; bottom panels). Informative IgG probes included: Neurofilament (neuronal, column 1), GFAP (glial, column 2) and striational (skeletal muscle sarcomere, column 3), and patient serum (column 4). Only the neuronal cell line was immunoreactive with patient serum.

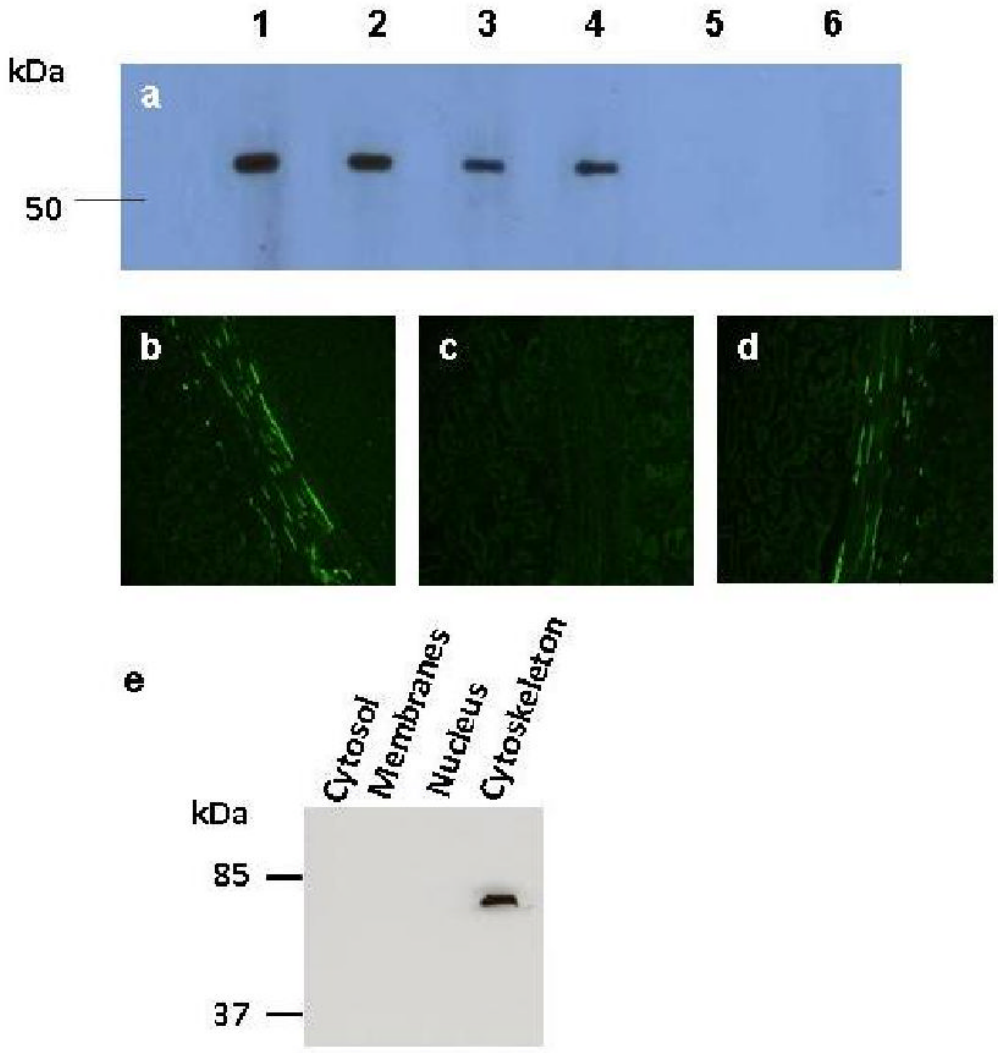

Fig. 3.

Novel IgG binds to a protein Mr-55-60kDa that is confined to the neuronal cytoskeleton. (a) Using PC12 lysate as a source of antigen, proteins were separated, immunoblotted, and probed with patient or normal human sera. A common band (~55kDa by reference to molecular weight standards) was revealed by IgG in patients' sera (lanes 1-4), but not by IgG in control human serum (lanes 5-7).

To verify specificity, patient IgG was affinity purified on the putative antigenic band and a control band. Eluates from the putative antigenic band (b) and control band (c) were reapplied to the composite mouse tissue substrate slide, and compared to the original immunostaining pattern of whole patient serum IgG on enteric nerve fibers (d). An identical staining pattern was observed in eluates from putative antigenic band. To determine the subcellular distribution of the antigen, PC12 lysates were fractionated by differential detergent extraction. Proteins in each fraction were resolved electrophoretically, transblotted, and probed with patient sera (e). IgG bound exclusively to a protein in the cytoskeletal fraction.

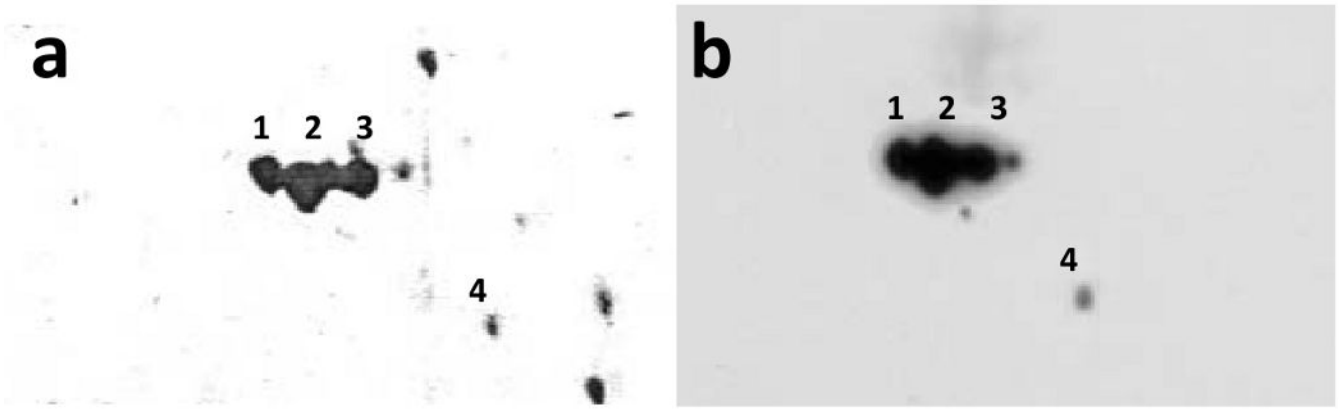

Fig. 4.

Identification of peripherin as the autoantigen. Duplicate preparations of the cytoskeletal fraction of PC12 cells were separated further by sequential isoelectric focusing and gel electrophoresis. One gel was silver stained (a), and the replica was transblotted and probed with patient serum (b). Unique peptides yielded by in-gel digestion from the silver stained gel of the common spots (numbered 1-4) identified the antigen to be peripherin.

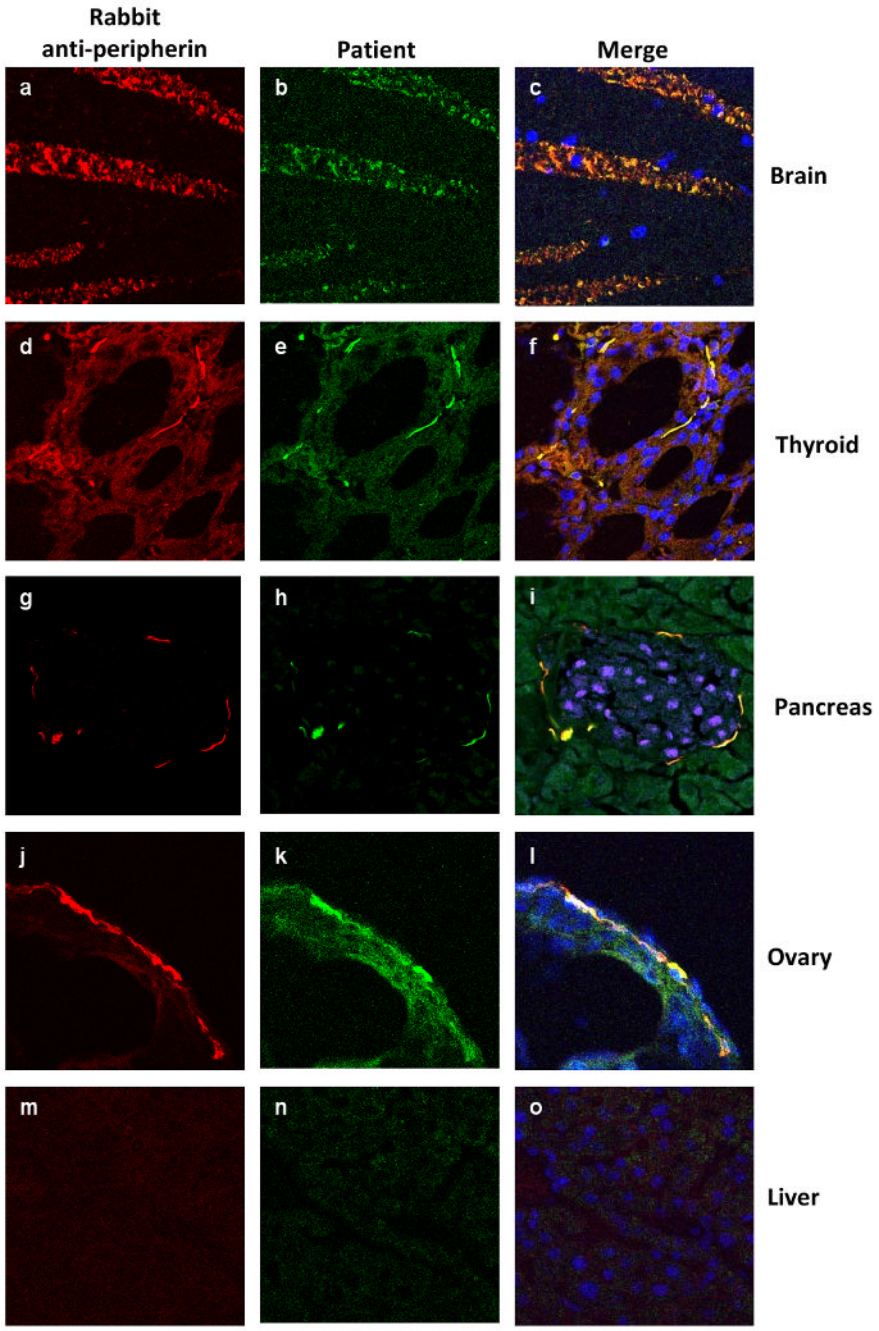

Fig. 5.

Patient IgG colocalizes with peripherin immunoreactivity in brain and endocrine organs. Tissues (brain [a-c], thyroid [d-f], pancreas [g-i], ovary [j-l] and liver [m-o]) were harvested from a 6-8 week old female mouse, cryosections (8 μm) were cut and stained with rabbit anti-peripherin-IgG (left columns), and patient IgG (center columns). All merged images (right column) show nuclear DAPI staining (blue) except for pancreas, where endocrine islet cells (i) were identified by IgG specific for the β-cell transcription factor PDX-1 (pseudo-colored purple).

Table 1

Clinical-serological correlations in 26 patients found incidentally to be seropositive for peripherin-IgG

Patient #	Age[a] (yrs)/sex/race	Peripherin IgG Titer, IF	Signs of dysautonomia			Other neurological symtoms or signs
			Generalized	Limited		
				Gastrointestinal	Other[b]	
1[c]	79/M/U	240	–	Achalasia, gastroparesis, weight loss	–	–
2[c]	60/F/C	240	–	Achalasia, weight loss	–	–
3[c]	38/F/C	1,920	–	Dysphagia, weight loss	–	–
4	26/F/AA	1,920	+	Gastroparesis, delayed bowel transit	ART abnormal	–
5[c]	46/F/C	3,840	–	–	Orthostatic hypotension; global anhidrosis (ART and TST abnormal); urinary urgency, incontinence	Numbness in legs, feet;
6[c]	39/F/C	240	–	Delayed gastrointestinal transit	–	–
7[c,d]	70/M/C	480	–	–	Orthostatic hypotension with syncope (ART abnormal)	Right homonymous hemianopia due to occipital metastasis[d]
8[c]	35/F/U	3840	–	–	ART consistent with postural orthostatic tachycardia syndrome (POTS)	Fatigue, diffuse muscle stiffness, numbness both hands and feet; fibromyalgia
9[c]	43/F/C	3,840	–	–	– (ART normal)	Numbness, tingling, painful feet and hands; dry eyes (lip biopsy consistent with Sjogren syndrome)
10[c]	40/F/U	1,920	–	–	–	Sensorimotor neuropathy
11	43/F/U	3,840	–	–	–	Optic neuropathy; peripheral neuropathy
12[c]	39/F/C	960	–	Delayed small bowel motility transit	– (ART normal)	Dizziness, palpitations
13[c,d]	70/F/C	1,920	–	– (weight loss only)	Mild orthostatic dizziness (ART and TST abnormal)	Left leg weakness, pain; left lumbosacral plexopathy; sensory-dominant peripheral neuropathy
14[c]	45/M/C	7,680	–	–	Erectile dysfunction	–
15[c]	47/M/C	1,920	–	–	–	Transverse myelitis (enhancing lesion at C5 level in spinal cord)
16[c]	46/F/C	3,840	–	–	–	Neck pain
17[c,d]	66/M/U	3,840	–	–	–	Myasthenia gravis
18[c]	47/F/C	960	–	Achalasia, weight loss	–	–

Patient #	Age[a] (yrs)/sex/race	Peripherin IgG Titer, IF	Signs of dysautonomia			Other neurological symtoms or signs
			Generalized	Limited		
				Gastrointestinal	Other[b]	
19	27/M/C	30,720	–	–	Mild orthostatism	Polyradiculoneuropathy (improvement followed with IVIG therapy)
20	59/M/C	7,680	–	–	–	Postural orthostatic tremor
21	47/M/C	7,680	+	Dysphagia, nausea, vomiting, weight loss	Erectile dysfunction. ART: small fiber neuropathy	
22	86/M/C	15,360	–	–	–	Cerebellar ataxia
23[d]	52/F/C	30,720	–	–	Hypotension	Anxiety disorder
24	40/F/H	7,680	–	–	–	Encephalitis with seizures
25[c]	21/M/C	3,840	–	–	–	Generalized whole body burning pain; hyperesthesia; low back pain; normal EMG. CSF protein elevated
26	60/F/C	30,720	–	–	–	Myelopathy

[a] Age at autoantibody detection. M, male; F, female. Ethnicities, C, Caucasian; AA, African American; H, Hispanic; U, unknown.

[b] Eight patients underwent autonomic laboratory evaluation of postganglionic sudomotor, cardiovagal, and adrenergic functions (ART, Autonomic Reflex Test; TST, Thermoregulatory Sweat Test).

[c] Mayo Clinic patients.

[d] Cancer detected: patient 7, adenosquamous cell lung carcinoma metastatic to left occipital lobe 8 years before autoantibody detection; patient 13, pituitary adenoma (panhypopituitarism followed resection); patient 23, breast carcinoma. Autoantibody profile in patient 17 (Table 2) was highly predictive of thymoma. [23]

Table 2

Endocrinopathies and co-existing autoantibodies in 26 patients with peripherin autoimmunity

Patient #	Age[a] (yrs)/sex/race	Diabetes		Other endocrinopathy	Co-existing autoantibodies	
		Onset age (yrs)	Insulin-dependence		Organ-specific	Non-organ-specific
1[b]	79/M/U	–	–	–	–	–
2[b]	60/F/C	–	–	–	–	–
3[b]	38/F/C	–	–	–	–	–
4	26/F/AA	22	Yes	–	GAD65 (6.03 nmol/L); IA-2 (0.39 nmol/L); VGCC, N-type (0.09 nmol/L)	ANA
5[b]	46/F/C	–	–	–	Thyroglobulin (1:400); thyroperoxidase (1:1600)	SMA
6[b]	39/F/C	–	–	–	–	–
7[b]	70/M/C	–	–	–	–	
8[b]	35/F/U	–	–	–	GAD65 (0.03 nmol/L)	–
9[b]	43/F/C	–	–	Hypothyroidism (age 43 yrs); premature menopause (age 38 yrs)	–	
10[b]	40/F/U	–	–	premature menopause (age 40 yrs)	–	–
11	43/F/U	–	–	–	–	–
12[b]	39/F/C	–	–	–	–	ANA
13[b]	70/F/C	70	No	Hypothyroidism; hypopituitarism*	–	–
14[b]	45/M/C	41	Yes	–	GAD65 (0.05 nmol/L); IA-2 (0.04 nmol/L); thyroglobulin (1:1600) thyroperoxidase (1:100)	–
15[b]	47/M/C	–	–	Hypothyroidism (age 48 yrs)	VGKC (0.06 nmol/L)	SMA
16[b]	46/F/C	–	–	–	–	–
17[b]	66/M/U	66	No	Hypothyroidism (age 61 yrs)	Muscle AChR (8.91 nmol/L); Striational Ab (1:61,440) CRMP-5-IgG (recombinant western blot)	ANA
18[b]	47/F/C	–	–	–	–	–
19	27/M/C	–	–	–	Ganglionic AChR (0.05nmol/L)	–
20	59/M/C	–	–	–	–	–
21	47/M/C	–	–	–	–	–

Patient #	Age[a] (yrs)/sex/race	Diabetes		Other endocrinopathy	Co-existing autoantibodies	
		Onset age (yrs)	Insulin-dependence		Organ-specific	Non-organ-specific
22	86/M/C	–	–	–	–	–
23	52/F/C	–	–	–	–	–
24	40/F/H	–	–	–	GAD65 (0.04 nmol/L) VGKC (0.39 nmol/L); thyroperoxidase (1:100)	–
25[b]	21/M/C	–	–	–	–	
26	60/F/C	–	–	–	–	–

[a] M, male; F, female. Ethnicities, C, Caucasian; AA, African American; H, Hispanic; U, unknown.

[b] Mayo Clinic patients.

[*] Post transphenoidal hypophysectomy due to non-functioning pituitary adenoma at age 57.

Table 3

Frequency of peripherin-IgG detection in archival sera of patient groups selected by clinical diagnostic categories

	Number of patients			Age, years[a]		Peripherin-IgG positive (%)	
Diagnostic group	**F**	**M**	**Total**	**Median**	**Range**	**Immunofluorescence**	**Western blot**
Diabetes, type 1	18	10	28	20	3-65	0	0
No neurological disorder							
Thymoma (7) or meningioma (1) with or without 1 or more endocrinopathies (type 1 diabetes, premature menopause or thyroiditis)	8	1	9	54	19-75	0	0
Small fiber neuropathy with/without autonomic involvement, premature menopause or type 1 diabetes	8	4	12	37	16-66	0	33

[a] Age at blood draw