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Ganglionic acetylcholine receptor autoantibodies in patients with Guillain-Barré syndrome

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ABSTRACT

Objectives: Although standardized autonomic tests are useful for diagnosing autonomic failure in patients with Guillain-Barré syndrome (GBS), they cannot be used as predictive markers. Thus, serological markers may correctly identify patients with GBS who are at risk for autonomic dysfunction.
Methods: We validated a luciferase immunoprecipitation system that detects IgG antibodies in patient serum that specifically bind to the α3 or β4 subunits of ganglionic neuronal nicotinic acetylcholine receptors (gAChR). We then used luciferase-conjugated ligands specific to antibodies against two gAChR subunits to test 79 sera samples from patients with GBS, 34 from subjects with other neurological diseases (OND), and 73 from healthy controls (HC). 1) In the first analysis, patients were classified into two groups according to the presence or absence of autonomic symptoms (AS). We compared the frequency of the anti-gAChR antibodies between these two groups (AS+ and AS−). 2) In the second analysis, furthermore, patients were classified depending on the presence or absence of anti-glycolipid antibodies (AGA). We compared the frequency of the anti-gAChR antibodies between the four categories of GBS (AS+/AGA+, AS+/AGA−, AS−/AGA+, and AS−/AGA−), OND, and HC.
Results: Eight subjects with GBS were positive for α3 subunits, while one was positive for β4 subunits. Anti-α3 and -β4 gAChR antibodies were also detected in 13.6% of AS+ GBS group in the first analysis. Two of 35 patients in AS− GBS group were seropositive for the anti-gAChR antibodies and AGA in the second analysis. Patients with GBS that were positive for serum antibodies to the α3 and/or β4 subunits of gAChRs showed a range of clinical features including AS and AGA.
Conclusions: Patients with GBS may have circulating antibodies against gAChR, which may contribute to the autonomic dysfunction associated with this disease.

1. Introduction

Autonomic involvement is an important and common complication of Guillain-Barré syndrome (GBS), with symptoms manifesting in the cardiovascular, sudomotor, gastrointestinal, and other systems; moreover, autonomic involvement can affect both sympathetic and parasympathetic fibers (van Doorn et al., 2008; Zochodne, 1994; Hughes et al., 2005). Studies on patients with GBS and animal models have provided a deeper understanding of the autonomic dysfunction that often accompanies this disease (Flachenecker, 2007; Zöllei et al., 2000; Ropper, 1994; Ropper and Wijdicks, 1990; Sakakibara et al., 2007). Previously, we reported that the clinical spectrum of GBS is associated with the reactivity of anti-ganglioside antibodies (Kaida et al., 2000; Kusunoki et al., 2008; Kusunoki and Kaida, 2011). We also analyzed the relationship between the severity of autonomic dysfunction and the profile of anti-ganglioside antibodies (Kuzumoto et al., 2006). However, it remains unclear whether a specific pathogenic factor for autonomic dysfunction is encountered in GBS. Thus, further exploration of new causative agents (e.g., other autoantibodies) of autonomic involvement in GBS is needed.

One promising area of study is the detection of autoantibodies to the ganglionic nicotinic acetylcholine receptor (gAChR), which mediates fast synaptic transmission in all peripheral autonomic ganglia (Vernino et al., 2009a). The properties of AChRs at mammalian

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ganglionic synapses are similar to the AChRs formed by the α3 and β4 subunits. In a recent study from our laboratory, we established a luciferase-reporter immunoprecipitation system (LIPS) assay for detecting the gAChR antibody, and were the first to report that patients with autoimmune autonomic ganglionopathy (AAG) have gAChR subunit-specific antibodies. More specifically, we reported that gAChR autoantibodies were detected in about 48% of patients with AAG (Nakane et al., 2015). Historically, cases with acute and subacute onset, often with antecedent infection and with a monophasic course, have been considered the autonomic equivalents of GBS and have been termed "acute pandysautonomia" or "autoimmune autonomic neuropathy" (Young et al., 1975). Although Klein et al. (Klein et al., 2003) noted that these entities was imprecise because some cases of AAG have an insidious onset and a slow progression, previous reports suggest that several atypical neuropathies have been included in the heterogeneous category of GBS (Uncini and Yuki, 2012; Seneviratne and Gunasekera, 2002; Pan et al., 2003). Therefore, in the present study, we extensively examined a sample of patients diagnosed with GBS in Japan. We then used a simple in vitro technique, i.e., the LIPS assay, to detect antibodies to the α3 or β4 subunits of gAChR. Using this method, we aimed to demonstrate the relationship between the clinical features of GBS, especially autonomic symptoms (AS), and autoantibodies (anti-glycolipid and anti-gAChR).

2. Methods

2.1. Participants, serum samples, and the enzyme-linked immunosorbent assay

Serum samples from patients with GBS in the acute phase were obtained from various general and teaching hospitals throughout Japan between June 2006 and June 2012. Patients with GBS were classified into two groups according to the presence or absence of AS in the first analysis. Next, in the second analysis, GBS patients were finally classified into the four categories depending on the presence or absence of anti-glycolipid antibodies (AGA) and AS, i.e., AS+/AGA+, AS+/AGA−, AS−/AGA+, and AS−/AGA− (Fig. 1). Thus, 79 serum samples were selected to evenly represent the four categories defined by the presence or absence of AS and AGA (Table 1). The clinical diagnoses and neurological findings (including AS) of the patients included in this study were determined at each hospital and provided to us at the time of our study; all patients met the diagnostic criteria of Asbury and Cornblath (Asbury and Cornblath, 1990). Serum antibodies against 10 glycolipid antigens (GalNAc-GD1a, GM1, GM2, GM3, GD1a, GD1b, GD3, GT1b, GQ1b, and Gal-C) were investigated using enzyme-linked immunosorbent assays (ELISAs), and these procedures have been described previously (Kaida et al., 2000; Samukawa et al., 2014). Here, 44 patients with GBS throughout Japan were identified by ELISAs as having immunoglobulin G (IgG) AGA. The control groups included in this study consisted of 73 healthy controls (HCs; mean age, 38.3 ± 11.1 years; 31 males and 42 females) and 34 subjects with other neurological diseases (ONDs; mean age, 56.3 ± 20.4 years; 19 males and 15 females).

Table 1
Clinical features of the patients with Guillain-Barré syndrome.

	AS (+)		AS (−)	
	AGA (+)	AGA (−)	AGA (+)	AGA (−)
Numbers	19	25	25	10
Age (years)	47.8 ± 21.9	49.2 ± 20.8	55.2 ± 16.8	38.7 ± 25.1
Sex				
Male (%)	14 (73.7)	15 (60.0)	14 (56.0)	6 (60.0)
Female (%)	5 (26.3)	10 (40.0)	11 (44.0)	4 (40.0)
Antecedent infections				
Respiratory tract (%)	12 (63.1)	13 (52.0)	17 (68.0)	3 (30.0)
GI tract (%)	6 (31.6)	9 (36.0)	7 (28.0)	6 (60.0)
Others (%)	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
No infection (%)	0 (0.0)	3 (12.0)	1 (4.0)	1 (10.0)

AGA, anti-glycolipid antibodies; AS, autonomic symptoms; GI, gastrointestinal.

2.2. Ethics

All subjects gave their written, informed consent to participate in the present study. This study was approved by the Ethics Committee of Nagasaki Kawatana Medical Center (Nagasaki, Japan) and the Kinki University Faculty of Medicine (Osaka, Japan).

2.3. LIPS assay for autoantibodies to gAChR

Serum antibodies to gAChRs were detected by the LIPS assay, as described previously (Nakane et al., 2015). To generate luciferase

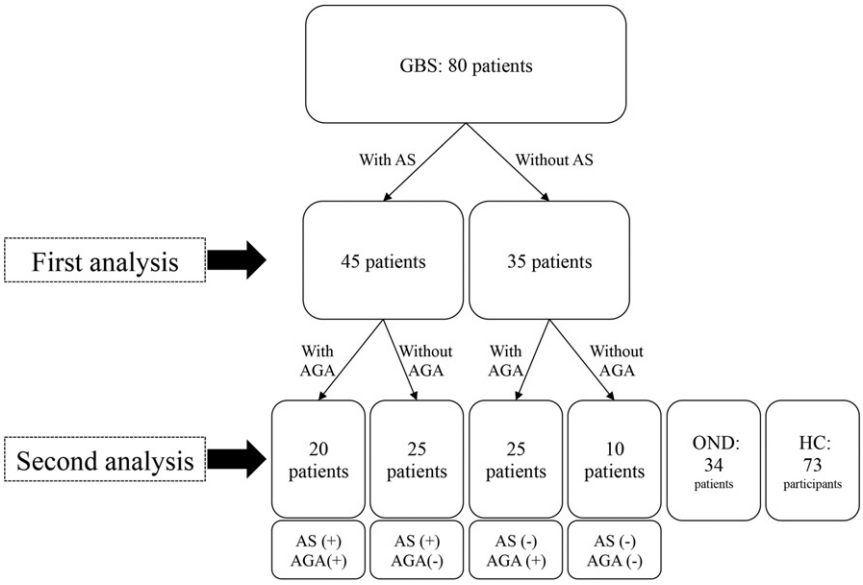

Fig. 1. Study design and analysis. Details regarding study design and recruitment for each subject group.