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Neural Autoantibody Evaluation in Functional Gastrointestinal Disorders: A Population-Based Case–Control Study

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Abstract

Background—Our goal is to investigate the serum profile of neural autoantibodies in community-based patients with irritable bowel syndrome (IBS) or functional dyspepsia. The pathogenesis of functional gastrointestinal (GI) disorders, including IBS and dyspepsia, are unknown. Theories range from purely psychological to autoimmune alterations in GI tract neuromuscular function.

Methods—The study subjects, based in Olmsted County, MN, reported symptoms of functional dyspepsia or IBS ($n = 69$), or were asymptomatic controls ($n = 64$). Their coded sera were screened for antibodies targeting neuronal, glial, and muscle autoantigens.

Results—The prevalence of neural autoantibodies with functional GI disorders did not differ significantly from controls (17% vs. 13%; $P = 0.43$). In no case was a neuronal or glial nuclear autoantibody or enteric neuronal autoantibody identified. Neuronal cation channel antibodies were identified in 9% of cases (voltage-gated potassium channel [VGKC] in one dyspepsia case and one IBS case, ganglionic acetylcholine receptor [AChR] in four IBS cases) and in 6% of controls (ganglionic AChR in one, voltage-gated calcium channel [VGCC], N-type, in two and VGKC in one; $P = 0.36$). The frequency of glutamic acid decarboxylase-65 (GAD65) autoantibodies was similar in cases (10%) and controls (5%; $P = 0.23$).

Conclusions—Our data do not support neural autoimmunity as the basis for most IBS or functional dyspepsia cases.

Keywords

Dyspepsia; Epidemiology; Irritable bowel syndrome; Autoimmune; Paraneoplastic

Introduction

Autoimmune gastrointestinal dysmotility (AGID) is a limited form of autoimmune dysautonomia with usually a subacute presentation of gastrointestinal (GI) hypermotility or hypomotility; pseudoobstruction is the most striking presentation [1]. AGID is recognized most frequently in the setting of serum autoantibodies reactive with neuronal ganglionic acetylcholine receptors (AChR containing $\alpha 3$ subunits) and Hu nuclear proteins (ANNA-1, also known as anti-Hu) [2-5]. Only ganglionic AChR antibodies have been implicated pathogenically. Rabbits immunized with ganglionic AChR and mice injected systemically with serum IgG containing ganglionic AChR autoantibodies develop severe dysautonomia, including slow GI transit [6]. Our laboratory has reported that the serum profiles of neural autoantibodies aid the diagnosis of AGID [1]. We recently reported that the frequency of glutamic acid decarboxylase-65 (GAD65) autoantibody is higher in achalasia patients than in healthy controls, suggesting an autoimmune basis for a subset of primary achalasia patients [7].

Functional GI disorders, including irritable bowel syndrome (IBS) and functional dyspepsia, are a poorly understood group of conditions characterized by unexplained upper and lower GI symptoms [8-12]. Both are disorders of exclusion, although symptom-based criteria can identify IBS with reasonable sensitivity and specificity [13-15]. Some reports suggest that IBS reflects a targeted immune attack on the enteric nervous system [16-19]. Törnblom et al. reported two patients with diarrhea-pre-dominant IBS commencing in adulthood who had, respectively, voltage-gated potassium channel (VGKC) and ganglionic AChR neuronal autoantibodies [16]. A recent review of the clinical correlations of VGKC antibodies that were detected incidentally in the Mayo Clinic Neuroimmunology Laboratory, in the course of service screening for paraneoplastic autoantibodies, reported amongst 17% of seropositive patients one or more symptoms of anorexia, weight loss, early satiety, nausea, vomiting, abdominal pain, bloating, diarrhea, or constipation [20]. In the present study, we comprehensively evaluated the frequency of serum autoantibodies targeting neuronal, glial, and muscle antigens in a population-based sample of subjects with functional GI disorders (functional dyspepsia and IBS) and asymptomatic controls.

Materials and Methods

Subjects

This study population has been described previously [21, 22]. We used the Rochester Epidemiology Project medical records linkage system to draw a random sample of 904 residents of Olmsted County stratified by age and sex (equal numbers of men and women) in 1990 [23]. Each subject was mailed a bowel disease questionnaire that has been shown to be a reliable and valid measure of GI symptoms [24]. It contained 42 questions that assess symptoms, including those of functional dyspepsia and IBS. Of the 904 people surveyed, 598 (66%) responded; 106 responding subjects were rejected (49 had a prior history of peptic ulcer disease/gastric surgery/organic GI or systemic disease; four had history of psychosis or mental retardation; 34 no longer resided in Olmsted County; one had died; 15 were pregnant; three for other reasons). Subjects aged 20–50 years who reported either dyspepsia (pain or ache above the navel) or IBS (pain or ache above or below the navel with

at least two of six Manning criteria symptoms) were considered as cases [8, 9, 25]. Those reporting no abdominal pain and less than two other GI symptoms on the bowel disease questionnaire were considered as controls. The Mayo Clinic medical record was reviewed for any neurological, gastroenterological, or oncological diagnosis.

Physician Interview

Of the 260 people meeting the inclusion criteria for the study, 152 (59%) came to the Clinical Research Center for a structured interview and an abdominal examination by a physician (GRL) blinded to the patients' diagnostic status. The remaining subjects underwent a telephone interview and were asked to participate by mail but did not provide blood specimens. All cases were classified into two groups:

1. Functional dyspepsia. Persistent or recurrent abdominal pain or discomfort centered in the upper abdomen, including postprandial fullness, early satiety, nausea, or upper abdominal bloating in the absence of a peptic ulcer history or other obvious cause [9, 21].
2. IBS. Persistent or recurrent abdominal pain or discomfort with at least two of the following six features: pain relief with bowel action, often; more frequent stools with pain, often; looser stools with pain, often; abdominal distention, often; sensation of incomplete rectal evacuation, often; or passage of mucus [9, 21, 25].

Those participants who came to the General Clinical Research Center were asked to provide a blood sample. Of the 152 who came in person, 146 consented and had a successful phlebotomy. The blood was centrifuged immediately and the serum placed into three vials. The initial evaluation included *Helicobacter pylori* serology, gastrin and pepsinogen measurement [22]. The remaining serum was frozen for future research studies, including a celiac disease serology study [21] and the present study.

Serological Analyses

All sera were tested blinded to the clinical diagnoses. Serum was available from 133 of 146 (91%) patients who provided a serum sample. We used the following assays to test for neural antibodies:

- a. Indirect immunofluorescence assay to test for neuronal nuclear and cytoplasmic autoantibodies (including ANNA-1, collapsin response-mediator protein [CRMP]-5-IgG, Purkinje cell antibody-type 2 [PCA-2]), peripherin IgG, or other novel IgGs binding to the enteric nervous system [2, 26-29]. Patients' sera were diluted (1:240) in PBS containing 1% BSA, pre-absorbed with beef liver powder, and applied to a composite substrate of adult mouse tissues (4- μ m-thick and postfixed with 10% formalin), including mouse stomach (mucosa and smooth muscle), kidney, and brain (cerebellum and brainstem). Figure 1 illustrates immunofluorescence staining patterns of several well-characterized neural autoantibodies recognized in our service laboratory to bind to enteric neural autoantigens (enteric ganglia or nerve trunks in the gut smooth muscle or nerve fibers in the mucosa and submucosa): ANNA-1 [2], CRMP-5-IgG [27], PCA-2 [28], and peripherin-IgG [29].
- b. Radioimmunoprecipitation assays to test for autoantibodies to neuronal VGKCs (α -dendrotoxin-sensitive), VGCCs (P/Q-type and N-type), nicotinic AChRs (both ganglionic-type [α 3 subunit-containing] and skeletal muscle-type), and glutamic acid decarboxylase-65 (GAD65) [30-33].
- c. Enzyme-linked immunosorbent assay (ELISA) to test for skeletal muscle striational (cytoplasmic) antibodies [20, 34].

- d. Western blot to test for CRMP-5-IgG (recombinant human protein) [35].

Clinical Follow-up

A detailed chart review was performed for cases or controls in whom a neural antibody was identified. The median duration of follow-up for these patients poststudy enrollment was 15 years (range 0–16 years).

Statistical Analysis

The proportion of subjects in each diagnostic subgroup with autoantibodies detected or not detected was evaluated by Fisher's exact tests. All *P*-values were two-tailed; the alpha level of significance was set at 0.05.

Results

Demographic data obtained in the initial cross-sectional survey for participants and non-participants were similar and have been published previously [21]. A separate demographic analysis was not done for the 133 patients that remained for this current study. The study group comprised 69 cases with functional GI disorders and available archival serum (functional dyspepsia in 34, IBS in 30, and both IBS and functional dyspepsia in five) and 64 asymptomatic controls. As reported previously, women were more frequent in the IBS symptom group than in the control group (71% vs. 48%; $P < 0.05$) [21]. Subjects with functional GI disorders were younger than controls (median age 31 vs. 39 years; $P < 0.05$).

The autoantibody frequencies and range of values in cases and controls are shown in Table 1. The prevalence of neural autoantibodies in subjects with functional GI disorders did not differ significantly from controls (17% vs. 13%, respectively; $P = 0.43$).

Immunofluorescence screening on a composite of mouse neural and non-neural tissues (Fig. 1) did not reveal any recognized neuronal or glial nuclear or cytoplasmic IgG or novel enteric neuronal IgG binding patterns.

Immunoprecipitation assays revealed neuronal cation channel antibodies in 9% of cases (VGKC, one with dyspepsia and one with IBS; ganglionic AChR, four with IBS) and in 6% of controls (ganglionic AChR, one; voltage-gated N-type calcium channel, two; VGKC, one; $P = 0.36$). Levels of cation channel antibodies, when detected, were low (<0.12 nmol/l), except for one dyspepsia case who had a VGKC antibody level of 0.27 nmol/l. Muscle AChR antibodies were not found. GAD65 and striational autoantibody frequencies were similar in subjects with functional GI disorders (10% and 1%, respectively) and in controls (5% in both; $P = 0.23$ and $P = 0.27$).

The clinical and oncologic associations for seropositive cases and controls are shown in Table 2. No case or control seropositive for any neural antibody developed neurological autoimmunity or GI dysmotility after a median duration of 15 years follow-up. However, neoplasia was diagnosed in 7 of 20 seropositive patients (35%) in the interim.

Discussion

Functional GI disorders affect 10–20% of the general population worldwide. The pathophysiology of these disorders is poorly understood, there are no disease-specific biomarkers, and diagnosis depends solely on symptom-based criteria. The concept that inflammation plays a role in IBS has raised interest in the immune system as a source of biological markers for this condition [36]. Several studies have focused on cytokines as indicators of immune activation. Some have suggested that a targeted autoimmune attack on neural-specific autoantigens may be an important but relatively ignored etiology [16].

In this community-based sample of cases with functional GI disorders, the frequency and levels of neural autoantibodies were low, and not significantly different from asymptomatic controls. Immunofluorescence screening revealed no well-characterized neuronal or glial nuclear or cytoplasmic autoantibodies in any subject, nor any novel IgG binding to enteric neurons in the stomach wall.

We recently reported that clinic-based patients with primary achalasia had an 11-fold higher frequency of GAD65 autoantibodies compared with healthy controls, supporting an organ-specific autoimmune basis in that disorder [7]. By contrast, in this population-based study, the frequencies of GAD65 autoantibodies were similar in functional dyspepsia and IBS cases and controls. Clinical follow-up for nine GAD65 antibody-positive subjects (six patients and three controls) revealed that two had diabetes. The frequency of this autoantibody was similar to that reported in the general adult population of Olmsted County (aged 50 years or older) [37].

Four cases and one control had low levels of ganglionic AChR autoantibodies (<0.11 nmol/l), which are a proven cause of autoimmune dysautonomia, both subacute and insidious in onset [38]. Ganglionic AChR antibodies were recognized initially in the context of idiopathic or paraneoplastic dysautonomia, generalized or limited (values typically exceeding 1.0 nmol/l) [5, 39, 40]. Since introducing the ganglionic AChR antibody assay into the standard Mayo Clinic serological evaluation for paraneoplastic autoimmunity (January 2005), we have observed broader oncological and neurological associations. Serum levels in positive patients are generally lower than 1.0 nmol/l [38]. Of patients with antibody values 0.10 nmol/l or higher, more than 82% have clinical evidence of an autoimmune neurological disorder. By contrast, 54% of patients with lower antibody values (0.03–0.09 nmol/l) lack evidence of an autoimmune neurological disorder. However, 30% of seropositive patients, regardless of the serum level, have an associated cancer (often adenocarcinoma) or a history of cancer [38]. Levels of ganglionic AChR antibodies were low in all seropositive cases of the present study, and no case developed symptoms of limited dysautonomia or other neurological problem in the follow-up after initial enrolment (up to 15 years). However, a cervical carcinoma in situ was found in one of five seropositive subjects in the course of follow-up evaluation at the Mayo Clinic.

The frequency of VGKC antibodies was less than 3% in both patients and controls, and in two of three seropositive patients, the serum levels were low (<0.06 nmol/l). The third patient was treated for a dermatofibroma. No seropositive patient developed symptoms of dysautonomia over the follow-up period, but cancer was diagnosed in two of the three seropositive subjects (cervical carcinoma in situ and breast carcinoma), 10 and 6 years after enrollment. In a recent review of 72 patients in whom VGKC autoantibodies were found after presentation with neurological symptoms, a neoplasm was encountered in 33% patients and VGKC antibody detection led to the cancer diagnosis in a majority of those patients [20]. Whether these antibodies are associated pathophysiologically with the GI symptoms reported remains to be determined. It seems likely, however, that the antibody detected was predictive of the later detected neoplasms.

VGCC antibodies have been described in the context of autoimmune GI dysmotility and in association with solid neoplasms (particularly carcinomas of the lung, breast, uterus, and ovary) but no case was seropositive in this study [41, 42]. Two control patients were seropositive for VGCC antibodies. No follow-up information was available for the control patients.

There are many potential limitations of this study, including the retrospective nature of the study and the modest sample size drawn from a random sample of 940 Olmsted County

residents. The 66% participation and the fact that participants and non-participants were similar in age, sex, and marital status suggests that the data are representative of the population studied. It is possible that non-participation may have resulted in the underestimation or overestimation of neural autoantibody seroprevalence. However, given the community-based “questionnaire and consent mail out” approach, the 58% participation of eligible cases and controls was higher than expected. At the time of serological testing for this study, serum was still available for testing in over 90% of these cases. Given how closely our study population (at the time of enrollment) was similar to that of the US white population, the results are more informative regarding this population and should not be extrapolated to other ethnic groups. In addition, given the population-based sampling strategy, these people likely had mild symptoms. That is, healthcare-seeking was not necessary and, in fact, only half of the cases had sought care for their GI symptoms. Therefore, the absence of AGID cases is not surprising. AGID plausibly explains severe dysmotilities, but those dysmotilities are rare in the community.

In summary, our extensive serological evaluation revealed a low frequency of neural autoantibodies in a population-based cohort of IBS and functional dyspepsia cases. The frequency did not differ from the case–control frequency. When detected, antibody levels were low. In some cases and controls, the detection of neural autoantibodies seemed to be predictive of neoplasia, but the predictive value of these tests needs further study. We did not study patients with very severe functional GI syndromes and cannot exclude a role for neurological autoimmunity in a small subset [16, 43]. The lack of neurological symptom development (including dysautonomia) in the follow-up period suggests that neurological autoimmunity is unlikely to be a significant factor in the etiology of functional GI disorders in the community.

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Fig. 1. Characteristic staining patterns of IgG autoantibodies that bind selectively to elements of the enteric nervous system (anti-neuronal nuclear [ANNA-1] and anti-neuronal cytoplasmic [CRMP-5, PCA-2, and peripherin]; indirect immunofluorescence, 4-μm frozen sections of mouse stomach). The *yellow arrows* indicate ganglionic neurons in myenteric plexus and the *white arrows* indicate immunoreactive nerve trunks and fibers in smooth muscle and mucosa. *SM* smooth muscle

Table 1

Neural autoantibody frequency and range of values in cases and controls

Autoantibody detected	Functional GI disorder cases (<i>n</i> = 69) % positive	Range of values (nmol/l or titer)	Control subjects (<i>n</i> = 64) % positive	Range of values (nmol/l or titer)	Normal values/range (nmol/l or titer)
<u>Neural-specific</u>					
Nicotinic acetylcholine receptor					
Ganglionic neuronal	5.8	0.04–0.11	1.6	0.04	0.00–0.02
Skeletal muscle	0	–	0	–	0.00–0.02
Neuronal voltage-gated Ca ²⁺ channel					
N-type	0	–	3.1	0.06–0.11	0.00–0.03
P/Q-type	0	–	0	–	0.00–0.02
Neuronal voltage-gated K ⁺ channel	2.9	0.05–0.27	1.6	0.04	0.00–0.02
Neuronal or glial nuclear or cytoplasmic (including ANNA-1, CRMP-5)	0	–	0	–	Negative
GAD65	10.1	0.03–0.39	4.7	0.05–0.45	0.00–0.02
Striational	1.4	240	4.7	240–480	Negative
One or more	17.4	–	12.5	–	–

No autoantibody was significantly more frequent in subjects with functional GI disorders than in asymptomatic controls (*P* > 0.05, Fisher's exact test)

ANNA-1 type 1 antineuronal nuclear autoantibody, CRMP-5 collapsing response-mediator protein, GAD65 glutamic acid decarboxylase (65-kD isoform), GI gastrointestinal

Table 2

Clinical follow-up information for cases ($n = 12$) or controls ($n = 8$) seropositive for any neural autoantibody

Subject (functional GI disorder or control)	Age at 1993 enrollment/sex	Autoantibody detected (value)	New diagnosis at last follow-up	Neoplasm at last follow-up	Last follow-up date (year) and period from enrollment years
1. Dyspepsia	27/M	VGKC (0.27 nmol/l)	–	Dermatofibroma	5 (2000)
2. Dyspepsia	41/F	GAD65 (0.09 nmol/l)	–	Rectal adenoma	16 (2009)
3. Dyspepsia	27/F	GAD65 (0.15 nmol/l)	Hypothyroidism	–	16 (2009)
4. IBS	64/M	GAD65 (0.05 nmol/l)	Diabetes, polyclonal hypergammaglobulinemia	–	15 (2008)
5. IBS	33/F	Striational (240) and ganglionic AChR (0.11 nmol/l)	–	–	16 (2009)
6. IBS	28/F	GAD65 (0.39 nmol/l)	Gestational diabetes	–	5 (1998)
7. IBS	26/F	Ganglionic AChR (0.04 nmol/l)	–	–	–
8. IBS	48/F	VGKC (0.05 nmol/l)	–	Breast carcinoma (2000), no recurrence (2009)	16 (2009)
9. IBS	31/F	Ganglionic AChR (0.08 nmol/l)	–	Uterine cervix CIN I	16 (2009)
10. IBS	25/M	Ganglionic AChR (0.08 nmol/l), GAD65 (0.03 nmol/l)	–	–	–
11. IBS	32/F	GAD65 (0.12 nmol/l)	Fibromyalgia, premature ovarian failure, gastroesophageal reflux disease	–	15 (2008)
12. IBS	48/M	GAD65 (0.06 nM)	Allergic rhinitis, parotid sialadenitis	–	11 (2004)
13. Control	33/F	Striational (240)	Bipolar disorder	–	10 (2003)
14. Control	49/M	GAD65 (0.05 nM)	Hearing loss, hypothyroidism, Sicca syndrome	Metastatic squamous cell carcinoma (1996) unknown primary	16 (2009)
15. Control	40/F	Striational (480), GAD65 (0.45 nM)	Varicose veins	–	13 (2006)
16. Control	52/M	Ganglionic AChR (0.04 nM)	No clinical information available	–	Died in 1995
17. Control	24/F	VGKC (0.04 nM)	–	Cervical intraepithelial neoplasia gr I; posthysterectomy (2004)	16 (2009)
18. Control	53/F	Striational (240)	Goiter	Invasive ductal breast carcinoma gr IV (1997)	13 (2006)
19. Control	30/M	VGCC-N-type (0.10 nM), GAD65 (0.05 nM)	–	–	13 (2006)
20. Control	30/M	VGCC-N-type (0.06 nM)	–	–	16 (2009)

ANNA-1 type 1 anti-neuronal nuclear autoantibody, *CRMP-5* collapsin response-mediator protein, *GAD65* glutamic acid decarboxylase (65-kD isoform), *GI* gastrointestinal, *IBS* irritable bowel syndrome, *VGCC* voltage-gated calcium channel, *VGCC* voltage-gated potassium channel