Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2010 July ; 51(1): 14–18. doi:10.1097/MPG.0b013e3181c15f78.

Antibodies to CBir1 are Associated with Glycogen Storage Disease Type Ib

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Abstract

Objectives—Glycogen storage disease (GSD) type Ib is a congenital disorder of glycogen metabolism that is associated with neutropenia, neutrophil and monocyte dysfunction, and an inflammatory bowel disease (IBD) that mimics a Crohn's disease phenotype. The enteric microflora is implicated in the pathogenesis of IBD; however, its role in the development of GSD-associated IBD is unknown. Antibody reactivity to *Saccharomyces cerevisiae* antibodies (ASCA), *Escherichia coli* outer membrane porin C (anti-OmpC) and bacterial flagellin (anti-CBir1) have been associated with Crohn's disease in the general population, but they have an undetermined association in children and adults with GSD-Ib. Our goal was to examine the association of ASCA, anti-OmpC and anti-CBir1 with the clinical features of GSD-Ib enterocolitis.

Methods—A retrospective review identified 19 GSD-Ib patients with or without a known diagnosis of enterocolitis. Radiographic, endoscopic and serologic data were collected and assays for ASCA, anti-OmpC, and anti-CBir1 obtained.

Results—Seven patients had combined radiographic, endoscopic, and histological evidence of intestinal inflammation; the majority had ileocolonic involvement. Seventeen of 19 (89%) patients had elevated anti-CBir1 levels (6/7 in the IBD group and 11/12 in the no clinical evidence of IBD group). Thirteen of 19 (68%) had elevated anti-OmpC levels (5/7 in the IBD group and 8/12 in the no clinical evidence of IBD group). Eleven of 19 (58%) patients had elevated ASCA IgA levels (4/7 in the IBD group and 7/12 in the no clinical evidence of IBD group).

Conclusion—Nearly all of the GSD type Ib patients had elevated anti-CBir1 levels. The antibody did not differentiate those with and without a diagnosis of GSD-Ib-associated IBD. Seroreactivity to flagellin may represent immune dysfunction rather than active enterocolitis in this patient population. Long-term follow-up of the group without known IBD is required to determine if these antibodies can predict intestinal inflammation.

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Keywords

glycogen storage disease type 1b; inflammatory bowel disease; antimicrobial reactivity

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Introduction

Glycogen storage disease (GSD) is frequently associated with intestinal inflammation that can involve any part of the gastrointestinal tract. GSD type I is a rare autosomal recessive disorder of glycogen metabolism that affects approximately 1 in 100,000 live births (1). The two major types are GSD-Ia and GSD-Ib. GSD-Ia is caused by deficiency of glucose-6-phosphatase while GSD-Ib is caused by a deficiency in the glucose-6-phosphate transporter (G6PT) which is required for the substrate to gain access to the enzyme (1). Diagnosis of this disorder is usually made during infancy when children present with episodes of fasting hypoglycemia, lactic acidosis, hyperlipidemia, and hepatomegaly. Other features of type I GSD include stomatitis, growth retardation, osteopenia, and hyperuricemia. GSD-Ib is similar to GSD-Ia with the added features of neutropenia and both neutrophil and monocyte dysfunction (2).

GSD is similar to certain subgroups of Crohn's disease in that there is a genetically derived innate immune defect, which may be associated with a dysregulated adaptive immune response. Up to 77% of GSD-Ib patients develop intestinal inflammation that can be clinically and histologically indistinguishable from Crohn's disease (CD) (3). Patients with GSD type Ib are frequently treated with granulocyte colony-stimulating factor (G-CSF). Some of those with a Crohn's-like enterocolitis respond to G-CSF while others require additional therapies such as 5-aminosalicylic acid derivatives, steroids and TNF-alpha inhibitors (4).

The presence of antibodies to yeast and bacterial antigens in the serum of CD patients implicate the role of endogenous fecal flora in the pathogenesis of IBD (5). Abnormal immune responses to the enteric microflora have led to the identification of anti-*Saccharomyces cerevisiae* antibodies (ASCA), anti-outer membrane porin C (OmpC) and anti-bacterial flagellin (anti-CBir1) antibodies in people with IBD (6,7). These antibodies can assist in the diagnosis and differentiation of CD from ulcerative colitis (5), and they can predict phenotypes and the natural history of CD (8). The presence of elevated ASCA has been reported in GSD-Ib patients (9) and in other immunodeficiency syndromes such as chronic granulomatous disease (10) and cystic fibrosis (11). The relationship and presence of anti-CBir1 and anti-OmpC antibodies in GSD patients with and without intestinal inflammation are unknown.

The majority of GSD-Ib patients have a wide array of gastrointestinal symptoms that can mimic IBD, even without the presence of active enteritis or colitis. Diagnostic testing can be difficult in this population given their tenuous metabolic state and young age making less invasive testing a necessity in these patients. A commercially-available assay (Prometheus IBD Serology 7) has been developed that measures ASCA, anti-OmpC, anti-CBir1, and perinuclear anti-neutrophil cytoplasmic antibody (pANCA) titers from the serum. The prevalence of these antibodies in GSD-Ib patients is unknown. The aim of this study was to identify and describe the serologic, clinical, radiographic, and histologic features of patients with GSD-Ib-associated IBD and to determine the association of the presence of ASCA, OmpC and CBir1 antibodies in GSD-Ib patients with a diagnosis of IBD and without a known diagnosis of IBD.

Materials and Methods

Patients

The clinical records of all patients with a diagnosis of GSD type Ib, who were evaluated at the Glycogen Storage Diseases Center at the University of Florida between August 2006 and April

2008 were reviewed. All research related activities were approved by the University of Florida Institutional Review Board. A total of 19 patients with GSD-Ib were identified.

Data collection

Clinical data that included gastrointestinal symptoms, recent medications, endoscopy results, histology results, laboratory values and radiographic studies were obtained by querying an IRB approved database that has been created to follow the GSD population. While all participants are followed by the GSD program at the University of Florida, not all of the testing was performed at the institution. Some patients already had ASCA IgA, ASCA IgG, anti-OmpC IgA, anti-CBir1 and pANCA antibody values determined by Prometheus labs as part of standard clinical care. Those patients that did not undergo this testing for clinical indications were consented to obtain the additional testing, and their serum was sent to Prometheus Laboratories (San Diego, CA). The serologic assays measured antibody titers by enzyme linked immunosorbent assay (ELISA) and values were reported as ELISA units (EU) per milliliter (mL). According to Prometheus Laboratories, the reference ranges were determined via a laboratory standard from a pool of patient sera. Reference values are reported as follows: ASCA immunoglobulin (Ig) A <20EU/mL, ASCA IgG <40EU/mL, anti-OmpC IgA <16.5EU/mL, anti-CBir1 <21EU/mL, and pANCA <12.1EU/mL.

Results

Patient Demographics

Nineteen patients were analyzed, 7 with a known diagnosis of GSD associated enterocolitis and 12 without. The median age at IBD diagnosis was 14 years (2–34 years), however many had symptoms predating the diagnosis. The IBD group was composed of 2 males and 5 females. The group without IBD was composed of 4 males and 8 females with a median age of 13 years (0.8–32 years). The clinical, radiographic, endoscopic, and histological features for those patients with GSD-Ib and intestinal inflammation are summarized in Table 1. The majority of patients without a known diagnosis of IBD did not undergo complete testing to evaluate for IBD as it was not clinically indicated, and any available test results are summarized in Table 2. Any imaging and endoscopic studies performed in the group without a diagnosis of IBD was for evaluation of transient symptoms of abdominal pain and/or less than four loose bowel movements per day. These symptoms were attributed to corn starch supplementation.

Clinical Features

Abdominal pain and diarrhea (more than 4 loose stools per day) were reported in all patients with GSD and IBD. None of the non-IBD patients had persistent abdominal pain or more than 4 loose stools daily. All patients were on cornstarch supplemented diets to treat the underlying metabolic derangements. Neutropenia predated the diagnosis of IBD in all subjects, and all patients were on G-CSF at the time of diagnosis of IBD. Stomatitis was reported in all patients (with and without IBD), but there were no reports of fistula, stricture or IBD-related surgery. All patients were started on an oral mesalamine at the time of IBD diagnosis. One patient had refractory diarrhea and abdominal pain that was treated with infliximab, which was later switched to adalimumab after she developed an infusion-related rash. Approximately one year after beginning adalimumab, a flat colonic polyp with high grade dysplasia was discovered in the setting of chronic active colitis during a surveillance colonoscopy.

Radiographic Findings

For the group of patients with GSD-associated IBD, there was no radiographic evidence of IBD in four of seven patients in whom abdominal/pelvic computed tomography and/or contrast radiography were performed. Barium meal small bowel series for two out of six patients were

abnormal. A tagged white blood cell scan showed abnormal small bowel uptake in the only patient that had the test.

Endoscopic and Histological Findings

All seven patients with a diagnosis of IBD had endoscopic evidence of mucosal inflammation ranging from erythema to obvious ulcerations. Review of the histology confirmed inflammation in the ileum and/or colon in 5 out of 7 patients. One 16 year old male with normal colon and ileal biopsies had gastric ulcerations with a Warthin-Starry stain that was negative for *Helicobacter pylori*. The other patient had evidence of small bowel inflammation seen on capsule endoscopy but an enteroscopy was not pursued. No granulomas were identified in any of our patients.

Immune Response

Review of the antibody levels demonstrated that anti-CBir1 was measurable in all patients with GSD-Ib and did not differentiate the two groups (Tables 2 and 3). Anti-CBir1 levels were elevated above the Prometheus provided reference range in 6 out of 7 (86%) patients with IBD with a mean value of 109.3 EU/ml and in 11 out of 12 (92%) patients without IBD with a mean value of 77.6 EU/ml. Anti-OmpC was elevated in 5 out of 7 patients (71%) with IBD with a mean value of 49.2 EU/ml and in 8 out of 12 patients (67%) without a diagnosis of IBD with a mean value of 40.5 EU/ml. ASCA IgA titers were elevated in 4 out of 7 (57%) with IBD with a mean value of 45.1 EU/ml and in 7 out of 12 (58%) without IBD with a mean value of 51.5 EU/ml. Interestingly ASCA IgG and pANCA titers were not prevalent in this patient population. An elevated ASCA IgG titer was identified in two patients (17%) without a diagnosis of IBD and in no patients with IBD. An elevated pANCA was discovered in two patients (29%) with IBD and in one patient (8%) without a diagnosis of IBD.

Discussion

This is the first report of a complete panel of ASCA, OmpC, CBir1 and pANCA seroreactivity in patients with GSD Ib. Seroreactivity to these antigens implicate a role for enteric microflora in the setting of a dysregulated immune response as reported in Crohn's disease (5). Thus, seropositivity to these antigens in GSD Ib patients may implicate a role for enteric microflora in the setting of G6PT deficiency, neutropenia and metabolic derangements. However, the presence of these antibodies did not differentiate those patients with or without GSD associated enterocolitis. In our cohort, 86% of GSD Ib patients with IBD had elevated anti-CBir1 titers and 71% had elevated anti-OmpC titers versus 92% and 67%, respectively, in those patients without known IBD. Presence of more than one antibody did not separate the groups. Both anti-OmpC and anti-CBir1 were elevated in 71% of GSD Ib patients with IBD and 67% without known IBD.

A weakness of our study is the lack of endoscopic and histologic data in the group without a known diagnosis of IBD. However, it would not be ethical to expose children to invasive procedures without clinical indications. Continued follow up of the group without known IBD will be important since these antibodies may predict progression of disease in Crohn's disease (8). We observed a significant disparity between ASCA IgA and IgG levels in both the IBD and no known IBD groups, which was not seen in the study by Melis et al because the Prometheus ASCA IgG cut off for a positive test was 40 EU/ml (9). The two patients that had low anti-CBir1 titers also had low anti-OmpC titers.

Given the high rate of seroreactivity in our patient population we did not find a correlation with clinical features. Up to 77% of GSD-Ib patients develop a Crohn's-like phenotype (3) and an evaluation for IBD should be considered in all patients with GSD-Ib and intestinal complaints.

Nonetheless, the diagnosis of IBD may be elusive in this rare metabolic disorder. Uncooked cornstarch diets for GSD type I may lead to symptoms such as diarrhea, abdominal distension and flatulence which may mask a diagnosis of IBD. Further, GSD-associated IBD may be unintentionally treated by G-CSF used for neutropenia. Screening endoscopic evaluation of GSD type Ib patients without high suspicion for IBD is generally avoided due to risk of hypoglycemia and lactic acidosis. Noninvasive testing such as CT scans and barium studies were negative in 57% of patients with IBD implicating the need for more sensitive and specific testing. Anti-CBir1 testing was introduced in 2006 and has proven useful for predicting fibrostenotic and complicated small bowel CD (12). However, even with the addition of this antibody, this test could not differentiate between those with and without IBD. It is entirely plausible that the group of patients without known IBD have low level intestinal inflammation that is undiagnosed. If that were the case, then diagnostic endoscopy and/or small bowel evaluation in patients with positive serologies would be warranted. Alternative noninvasive testing for fecal proteins such as S100A12 and calprotectin, may also be a sensitive and specific means of identifying IBD (13) and could also be evaluated in our patient population.

Even though it is unclear if the presence of anti-CBir1 is of clinical significance in this patient population, it is noteworthy to point out that the levels were quite elevated in nearly all the patients. The results may not be coincidental as anti-CBir1 is associated with disease in the small intestine (12), which is the region most commonly affected in GSD-associated enterocolitis. Alternatively, these GSD-Ib patients are maintained on cornstarch-supplemented diets, which could potentially skew the composition of the enteric flora; and when superimposed on a background of immune dysfunction, it could exacerbate anti-flagellin responses.

In summary, positive IBD serologies are almost universally present in GSD-Ib. Our findings make it plausible that aberrant immune responses to certain antigens are associated with the immune dysfunction of GSD Ib. While these studies may be prognostic for future GSD-associated enterocolitis, they should not be considered diagnostic of the condition. All GSD-Ib patients with intestinal symptoms should undergo diagnostic testing to rule out the presence of intestinal inflammation.

Acknowledgments

Support: These investigations were supported in part by NIH General Clinical Research Center Grant M01 RR 00082 (Florida) and a grant from Prometheus Laboratories Inc., California. DW was supported by Mentored Career Award K23 RR 017560. SP was supported in part by Crohn's and Colitis Foundation of America Career Development Award.

Abbreviations

GSD	Glycogen storage disease
IBD	inflammatory bowel disease
OmpC	<i>Escherichia coli</i> outer membrane porin C
CBir1	CBir1 flagellin
ASCA	anti- <i>Saccharomyces cerevisiae</i> antibody
pANCA	perinuclear anti-neutrophil cytoplasmic antibody

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Table 1

Clinical features of patients with GSD-Ib and a diagnosis of IBD

Pt#	Gender and Age (yrs)	Medical History	Symptoms	Recent Medications	Colonoscopy and EGO	Histology	Radiographic Studies
1	F27	- GSD-Ib - Pancreatitis - IBD diagnosed age 11	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine - Budesonide	- Transverse colon ulcers - Terminal ileum ulcers - EGD ND	Chronic active ileitis and colitis	- UGI/SBFT abnormal terminal ileum and cecum - CT A/P thickened terminal ileum and cecum
2	F29	- GSD-Ib - IBD diagnosed age 20	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine	- Ascending colon erythema - Terminal ileum normal - EGD ND	- Chronic active colitis - Nonspecific changes in ileum	- UGI/SBFT normal - Tagged WBC scan increased small bowel uptake
3	M16	- GSD-Ib - Liver adenoma - IBD diagnosed age 14	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine	- Entire colon patchy erythema - Terminal ileum normal - Gastric ulcers	- Colon and ileum normal - Chronic active gastritis	- CT A/P normal - UGI/SBFT normal
4	F21	- GSD-Ib - Pancreatitis - IBD diagnosed age 17	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine - Budesonide	- Colon normal - Terminal ileum ulcers - EGD ND - Scattered small bowel ulcers by video capsule endoscopy	Colon and ileum normal	CT A/P thickened terminal ileum

Pt#	Gender and Age (yrs)	Medical History	Symptoms	Recent Medications	Colonoscopy and EGO	Histology	Radiographic Studies
5	F24	- GSD-Ib - IBD diagnosed age 8	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine	- Entire colon patchy erythema - Terminal ileum normal - EGD ND	- Chronic active colitis - Ileum normal	- CT enterography normal - UGI/SBFT normal
6	F36	- GSD-Ib - IBD diagnosed age 34	- Abdominal pain - Diarrhea - Perirectal abscess - Stomatitis	- G-CSF - Mesalamine - Adalimumab	- Entire colon patchy erythema and ulcers - EGD ND	- Chronic active colitis - Focal active ileitis	CT enterography normal
7	M19	- GSD-Ib - Appendectomy - IBD diagnosed age 10	- Abdominal pain - Diarrhea - Stomatitis	- G-CSF - Mesalamine	- Ascending colon patchy erythema - Terminal ileum normal - EGD ND	- Chronic active colitis - Ileum normal	UGI/SBFT abnormal terminal ileum and cecum

Computed tomography (CT) scans were performed with oral and intravenous contrast. CT A/P, CT abdomen and pelvis; EGD, esophagogastroduodenoscopy; ND, not done; UGI/SBFT, upper gastrointestinal series with small bowel follow through; WBC, white blood cell.

Table 2
Clinical features and serum antibody levels in GSD-1b patients without a known diagnosis of IBD

Pt#	Gender and Age (yrs)	IBD studies	ASCA IgA (EU/ml)	ASCA IgG (EU/ml)	Anti-OmpC IgA (EU/ml)	Anti-cBir1 (EU/ml)	ANCA (EU/ml)
8	F20	ND	65.8	<12	17.3	39.9	<12.1
9	M15	ND	34.2	<12	64.8	100	<12.1
10	F6	ND	39.9	65.3	8.6	46.1	<12.1
11	F20	Normal colonoscopy	<12	19.9	27.6	130.8	<12.1
12	F15	ND	13.5	29	93.1	49.4	<12.1
13	M13	Normal colonoscopy and CT	<12	<12	20.6	103.5	<12.1
14	M14	Normal CT	36.4	14.8	17.7	83.4	<12.1
15	F1	Normal UGI/SBFT	104.8	70	22.6	125.4	23.1
16	F2	ND	<12	<12	37.3	27.9	<12.1
17	F0.8	ND	28.9	33.4	8.3	93.9	<12.1
18	M2	ND	12.4	<12	1.1	52.8	<12.1
19	F32	ND	50.2	22	10.5	15.5	<12.1

CT, computed tomography with intravenous and oral contrast; UGI/SBFT, upper gastrointestinal series with small bowel follow through; ND, not done. Reference range values: ASCA IgA <20 EU/mL, ASCA IgG <40 EU/mL, anti-OmpC IgA <16.5 EU/mL, anti-cBir1 <21 EU/mL, and pANCA IgA <12.1 EU/mL.

Table 3

Serum antibody levels in GSD-1b patients with a diagnosis of IBD

Pt#	Disease Location	ASCA IgA (EU/ml)	ASCA IgG (EU/ml)	Anti-OmpC IgA (EU/ml)	Anti-CBir1 (EU/ml)	ANCA (EU/ml)
1	Ileocolonic	<12	<12	7.8	87.7	<12.1
2	Ileocolonic	<12	<12	78	115.7	24.9
3	Gastric	30.5	23.6	56.5	113.8	<12.1
4	Ileal	30.8	13.6	24.7	122.2	<12.1
5	Colonic	25.4	30.7	37.5	110.2	22.2
6	Ileocolonic	93.8	25.3	>111	105.9	<12.1
7	Colonic	<12	<12	6.2	19.9	<12.1

Reference range values: ASCA IgA <20 EU/mL, ASCA IgG <40 EU/mL, anti-OmpC IgA <16.5 EU/mL, anti-CBir1 <21 EU/mL, and pANCA <12.1 EU/mL.