High Incidence of Serologic Markers of Inflammatory Bowel Disease in Asymptomatic Patients with Glycogen Storage Disease Type Ia

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Abstract Most patients with glycogen storage disease (GSD) type Ib show features related to inflammatory bowel disease (IBD). The development of IBD seems to be associated with the defect of neutrophil function in GSD Ib. Patients with GSD Ia were not recognized to have similar gastrointestinal complaints until recently and are not associated with a neutrophil defect. Fifty consecutive GSD Ia inpatients over the age of 2 years without a diagnosis of IBD were screened using serologic and genetic markers via the Prometheus IBD sgi Diagnostic test. Eleven patients were tested positive for IBD (22%), with five fitting the pattern for Crohn's disease, five for ulcerative colitis, and one with nonspecific IBD. Only 2 out of the 11 patients had any gastrointestinal complaints. No pattern could be distinguished from individual inflammatory markers, genetics, inflammation antibodies, age, complications, or metabolic control. Of note, 9 out of 11 patients testing positive were female. Patients with GSD Ia were found to have a higher rate of serologically indicated IBD when compared with the general population. While these subjects will need to be followed to determine if these serologic markers correlate with clinical disease, this study supports that IBD may be more common in the GSD Ia population. Further

studies are warranted to explain the relationship between IBD and GSD I since it may provide clues regarding the pathogenesis of IBD development in the general population.

Background

Glycogen storage disease type Ia (GSD Ia; OMIM 232200) is an autosomal recessive disorder of glycogen metabolism that affects approximately 1 in 100,000 live births (Chen 2001). Mutations of the genes that encode glucose-6-phosphatase (subtype Ia) and glucose-6-phosphate translocase (subtype Ib) have been isolated (Lei et al. 1993, 1995). Diagnosis is suspected in infants with fasting hypoglycemia, lactic acidosis, hyperlipidemia, and hepatomegaly. Other complications include hepatic adenomas, growth retardation, osteoporosis, and nephropathy. GSD Ib is similar to GSD Ia with the added features of neutropenia and phagocyte dysfunction (Gitzelmann and Bosshard 1993). Without immediate recognition of symptoms and appropriate treatment, GSD can be fatal in infancy. Fortunately, much success has been achieved in the management of GSD as blood glucose levels can be sustained with cornstarch and overnight nasogastric feeds (Wolfsdorf and Crigler 1997, 1999). However, the improved mortality rates have been offset by an increase in morbidity as patients reach older ages.

Roe et al. (1986) first described an association between inflammatory bowel disease (IBD) and GSD Ib. Since that time, there have been continued investigations on the causal relationship of these two conditions (Melis et al. 2003). Historically, neutropenia has been used to explain the almost universal development of GSD enterocolitis in patients with GSD Ib. Surprisingly, treatment with granulocyte colony-stimulating factor has led to an improvement of

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the symptoms of IBD, but has not resulted in the resolution of the disease (Visser et al. 2002). It is published that up to 77% of GSD Ib patients have serologically, histologically, and clinically confirmed IBD (Visser et al. 2000). A prospective study of 19 asymptomatic patients with GSD Ib also found that 89% of the subjects had elevated anti-CBir1 concentrations and serologic studies consistent with IBD (Davis et al. 2010). Until recently, there have been no definitive connections made between IBD and other types of GSD.

We have recently found a cohort of GSD Ia patients that are endoscopically and/or histologically confirmed to have either enteritis or colitis, two of which had characteristics of Crohn's disease (CD) and three with disease most consistent with ulcerative colitis (UC) (Lawrence et al. 2014). As mentioned previously, IBD in patients with GSD Ib is typically attributed to functional deficiency in neutrophils. However to date, GSD Ia is rarely associated with a quantitative or qualitative defect of neutrophils.

The aim of this study was to determine if there is a genetic or serologic predisposition of patients with GSD Ia to develop IBD.

Methods

Blood samples were obtained from 50 consecutive inpatients with GSD Ia who qualified for the study and did not already have a diagnosis of IBD at UF Health Shands Hospital between January 2014 and October 2014. Informed consent was obtained from all participants to confirm they understood how their sample would be used in an IBD panel. Study protocols conformed to ethical guidelines and was approved by the University of Florida Institutional Review Board (IRB201300312).

This is an observational, nonrandomized prevalence study of patients diagnosed with GSD Ia who did not manifest symptoms of IBD. The Prometheus IBD sgi Diagnostic test (Prometheus Laboratories, San Diego, CA) was used to predict and differentiate IBD based on an algorithm combining serologic, genetic, and inflammatory markers. The serologic markers include antibodies against yeast (ASCA-IgA and -IgG), antibodies against bacteria (Anti-OmpC, Anti-CBir1, Anti-Fla-X, and Anti-A4-Fla2), and autoantibodies (ANCA, pANCA, and DNase-sensitive ANCA). Genetic markers include *ATG16L1*, *NKX2-3*, *STAT3*, and *ECM1*. These are associated with autophagy, cell type specification and differentiation, signal transduction, and the extracellular matrix, respectively. Inflammatory markers include ICAM-1, VCAM-1, VEGF, CRP, and SAA.

To ensure appropriate statistical power, 50 patients were recruited as the total cohort. Means and standard deviations

were used to analyze the continuous data. Frequency tables were used to summarize categorical data.

Results

Out of 50 total participants in the study, 11 tested positive for IBD. Five had a pattern consistent with Crohn's disease, five had a pattern consistent with ulcerative colitis, and one had nonspecific IBD. Our prevalence rate is thus 22% within this cohort.

Of note, 9 out of 11 patients testing positive for IBD were female. Sex and panel result were not independent ($\chi^2 = 11.22$, $p = 0.007$). For all other individual variables (age, antibody assays, genetics/genotype, inflammatory markers), either no pattern was discovered or there was no significant difference between patients testing positive for IBD and patients testing negative for IBD (Fig. 1, Table 1). The same patients testing positive do not seem to show a higher complication rate or less optimal metabolic control than the patients testing negative.

Discussion

The management of GSD has markedly improved over the past 40 years. While survival was rare prior to the 1970s, most patients with GSD Ia and Ib are now doing well into adulthood (Rake et al. 2002; Visser et al. 2000). With aging, long-term complications have been appreciated, including the development of hepatic adenomas (Beegle et al. 2015), hepatocellular carcinomas (Limmer et al. 1988), osteoporosis (Minarich et al. 2012), and hepcidin-induced anemia (Wang et al. 2012). IBD may be a

Fig. 1 Inflammatory bowel disease (IBD) panel result of patients by age

Table 1 Serological, genetic, and inflammatory markers of patients who tested positive for inflammatory bowel disease (IBD)

Patient	1	2	3	4	5	6	7	8	9	10	11
Sex	F	F	F	F	F	M	F	F	M	F	F
Age (year)	13	6	7	22	10	18	9	10	13	11	31
Diagnosis	CD										
	UC										
	Inconclusive for CD vs. UC										
Serology	ASCA-IgA ELISA (EU/mL)	4.8	<3.1	<3.1	<3.1	<3.1	<3.1	<3.1	<3.1	<3.1	6.1
	ASCA IgG ELISA (EU/mL)	4.8	<3.1	<3.1	12.3	<3.1	8.4	8.8	6.2	5.1	<3.1
	Anti-OmpC IgA ELISA (EU/mL)	5.0	3.7	<3.1	<3.1	11.7	4.9	4.0	3.4	4.9	13.1
	Anti-CBir1 IgG ELISA (EU/mL)	>100.0	94.7	46.3	7.5	>100.0	>100.0	>100.0	40.0	21.5	63.7
	Anti-A4-Fla2 IgG ELISA (EU/mL)	77.7	55.9	91.9	8.8	76.6	79.8	36.8	11.9	22.6	82.9
	Anti-FlaX IgG ELISA (EU/mL)	>100.0	78.4	80.3	20.3	>100.0	>100.0	56.5	15.8	31.4	>100.0
	AutoAntibody ELISA (EU/mL)	18.6	22.1	29.4	34.4	53.0	6.7	<3.1	21.5	29.1	8.9
	IFA perinuclear pattern detected								x	x	
Genetics	DNase sensitive								x	x	
	ATG16L1 (rs2241880)	G;G	A;A	G;A	G;G	G;A	A;G	A;A	G;G	A;A	A;G
	ECM1 (rs3737240)	C;C	C;C	T;C	C;C	T;T	C;C	C;C	C;T	C;C	C;T
	NKX2-3 (rs10883365)	A;A	A;A	G;G	G;G	G;G	G;G	G;G	G;G	A;A	A;G
	STAT3 (rs744166)	A;G	A;A	G;G	A;A	A;A	A;A	G;G	A;A	G;G	A;A
Inflammation	ICAM-1 (µg/mL)	0.47	0.43	0.70	0.55	0.46	0.45	0.46	0.42	0.66	0.39
	VCAM-1 (µg/mL)	0.52	0.53	1.19	0.40	0.57	0.45	0.59	0.71	0.68	0.51
	VEGF (pg/mL)	812	244	401	664	844	576	171	391	884	553
	CRP (mg/L)	0.3	0.4	1.2	20.9	1.6	1.9	0.6	0.2	16.4	12.5
	SAA (mg/L)	2.2	1.1	4.6	13.8	2.5	2.7	1.4	1.4	1.9	11.7

complication of GSD Ia that has been recently uncovered. Our 22% prevalence rate dramatically exceeds the incidence of IBD in the general adult population in the United States, which is 0.91% (Nguyen et al. 2014). A comparable study of pediatric IBD prevalence in Canada found values of 0.02% for ages 0–9 and 0.15% for ages 10–19 (Benchimol et al. 2014). While it seems that there was a trend toward children to test positive for IBD since 9 out of 11 patients were under 20 years old, we were unable to include some GSD Ia adults in this study because they already had a diagnosis of IBD.

While an association between GSD Ib and IBD is well recognized, there are few reports focusing on the presence of diarrhea in the GSD Ia patient and none have linked this symptom to IBD. Unlike in patients with GSD Ib, patients diagnosed with GSD Ia do not have neutropenia to explain this consequence. Patients seen at the University of Florida and managed with cornstarch have frequently complained of adverse gastrointestinal effects, namely, abdominal pain and diarrhea, as has been previously reported at other institutions (Grabitske and Slavin 2009). As such, a possible explanation for our findings is that the IBD is secondary to the effects of cornstarch on the gastrointestinal tract. Cornstarch creates an acidic environment. In general, when the stools of nonhuman subjects consuming diets high in cornstarch are analyzed, they typically demonstrate increased reducing substances and a decreased pH (Kotarski et al. 1992). These findings are consistent with carbohydrate malabsorption. Fermenting bacteria in the colon are responsible for metabolizing carbohydrates that are not absorbed in the small intestine into short-chain fatty acids (SCFA). As a result, these SCFA are absorbed throughout the colon and, in addition, are involved in the reabsorption of water and sodium. The acidification of the stools leads to an alteration in the amount of fermenting bacteria, and thus there is decreased conversion of unabsorbable starches to SCFA. This can lead to significant diarrhea (Treem et al. 1996). While cornstarch may be involved in the pathogenesis of IBD development in GSD I, IBD has not been recognized in other types of GSD which also use this therapy. However, other types of GSD do not have doses of cornstarch therapy as high as in type I. We also have not yet seen a difference between uncooked cornstarch and the slow-release hydrothermally processed cornstarch which has become more popular for overnight use.

A second possible explanation for the development of IBD is that the underlying enzyme defect in GSD causes abnormalities that predispose to the development of IBD. Glucose-6-phosphatase is known to be present in the small intestine, but the function of the intestinal enzyme has not been elucidated. Davis et al. linked antibodies to CBir-1 to all the patients with GSD Ib who have IBD (Davis et al. 2010). The finding is significant because CBir-1 antibodies

are found in 50% of all patients with Crohn's disease and more prevalent in small bowel disease. In contrast to the GSD Ib population, colonic disease predominated in the GSD Ia population when compared with the GSD Ib population. This supports that the intestinal abnormality alone is not sufficient to cause the disease. The role of the intestinal defect will likely be elucidated by the studies in the intestinal glucose-6-phosphatase knockout mouse model (Rajas et al. 2014).

A third possibility is that the development of IBD is not directly related to GSD, but instead occurs due to a genetic or epigenetic predisposition of both diseases in certain populations. For example, the Ashkenazi Jewish population has a carrier rate of 1 in 72 for GSD, and this population also has a high risk of IBD (Ekstein et al. 2004). The strongest evidence for a genetic cause of IBD may be found in the human studies of twins (Halfvarson et al. 2003). Patients of Jewish descent are up to four times more likely to develop the disease. Moreover, it has been estimated that the rate of developing IBD is 20 times more likely in first degree relatives of Jewish patients with IBD (Yang et al. 1993). Several of the patients in this cohort carried the classic Jewish mutation for GSD Ia, placing them at higher risk at baseline for developing IBD. While this genetic predisposition should be considered, not all of the patients had Jewish heritage or the mutation associated with this population which supports that this indeed is a complication of GSD.

While the rate of positivity was markedly higher in the GSD population when compared with the general population, it is important to note that there are significant drawbacks to the Prometheus IBD sgi Diagnostic test as outlined previously: three of the least expensive and most available components may account for the entire predictive value of the test, and routine laboratory tests may have higher predictive values (Benor et al. 2010; Shirts et al. 2012). In addition, no endoscopies were performed to confirm the diagnosis of IBD on the patients who tested positive on the IBD panel because they were subclinical, and ethically the risk of the procedure was deemed too high. In GSD, endoscopies are not benign and require the patient to be admitted to the hospital in advance of the procedure to maintain glucose concentrations during the cleanout. During the procedure itself, crises can occur if the patient experiences excessive stress due to increased metabolic load or if the glucose infusion is interrupted. These patients should continue to be followed in the future to determine if they eventually develop IBD.

Conclusions

In light of finding an increased rate of serologic IBD in the GSD Ia population, it is clear that further research is needed

to characterize the relationship between IBD and GSD I. If patients testing positive for IBD develop clinical manifestations of the disease, the confirmation of a higher rate of IBD in GSD Ia may provide important clues to the pathogenesis of IBD in the general population.

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Synopsis

Serologic panels suggest inflammatory bowel disease may be a new complication of glycogen storage disease type Ia.

Compliance with Ethics Guidelines

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. The study was approved by the University of Florida Institutional Review Board (IRB201300312).

Details of the Contributions of Individual Authors

NL and DW conceived of and participated in the design of the study. NL, TC, and LB recruited participants for the study. TC performed the data collection and statistical analysis. NL and TC co-drafted the manuscript and LB and DW provided critical review. All authors read and approved the final manuscript.

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