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HLA-DQ Genotype Is Associated with Accelerated Small Bowel Transit in Patients with Diarrhea-Predominant Irritable Bowel Syndrome

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Abstract

Background—Colonic transit (CT) is accelerated in 46% of patients with diarrhea-predominant irritable bowel syndrome (IBS-D). Improvement in IBS-D with gluten withdrawal is associated with HLA-DQ2 positivity; the mechanism of improvement is unclear.

Objective—To determine if HLA-DQ2 or HLA-DQ8 positive IBS-D patients have faster small bowel (SB) or CT than HLA-DQ2 and HLA-DQ8 negative patients.

Methods—Among 94 IBS-D patients who previously provided DNA samples, 64 had undergone validated measurements of CT (geometric center at 24h [GC24]); 50 of the patients also had measurement of gastric emptying (GE) and 54 of SB transit (colonic filling at 6h [CF6h]). HLA-DQ status was determined by tag SNP approach. Associations of CF6h and GC24 with HLA-DQ2 and HLA-DQ8 status were assessed using analysis of covariance (ANCOVA), adjusting for BMI.

Results—Mean age was 40.8±1.6y; 98.5% were female. In 60/64 patients, celiac disease was excluded by serology or histology. There were no significant differences in age or BMI among the different HLA-DQ groups. Independently, patients positive for HLA-DQ2 had numerically greater CF6h compared to HLA-DQ2 negative (p=0.065), and those positive for HLA-DQ8 had greater CF6h compared to HLA-DQ8 negative patients (p=0.021). GE was not associated with HLA-DQ2 and HLA-DQ8 status. Patients positive for both HLA-DQ2 and HLA-DQ8 had greater CF6 (p=0.013) and numerically higher, but not significant, GC24 (p=0.38) compared to HLA-DQ2 and HLA-DQ8 negative patients.

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Conclusion—IBS-D patients positive for HLA-DQ8 or for both HLA-DQ2 and HLA-DQ8 have faster SB transit. The mechanism of the accelerated SB transit and the effect of gluten withdrawal on SB function in IBS-D deserve further investigation.

Keywords

HLA-DQ; small bowel transit; diarrhea-predominant irritable bowel syndrome

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic gastrointestinal condition characterized by recurrent abdominal pain or discomfort with altered bowel habits such as diarrhea. Approximately 46% of patients with diarrhea-predominant IBS (IBS-D) have accelerated colonic transit (1). It is known that a minority (up to 5%) of patients presenting with symptoms suggestive of IBS-D in community-based or referral centers has celiac disease (2). Some patients with IBS report an association of symptoms with specific food triggers, suggesting a role of food hypersensitivity (3–5). One of these reported food triggers is gluten in the absence of overt celiac disease.

Gluten sensitive diarrhea without celiac disease was first proposed as a clinical entity in 1980 (6) by Cooper and colleagues. The spectrum of gluten sensitivity ranges from minimal histological changes such as increased intraepithelial lymphocytes without villous atrophy, increased IgA deposits in intestinal villi, gluten sensitive diarrhea and immunological mucosal response to gluten exclusion in first-degree relatives of patients with celiac disease (7). Typically, one or more of these findings are seen in individuals who are positive for HLA-DQ2 or HLA-DQ8, suggesting that this entity may be immune mediated, as previously described (7, 8). Wahnschaffe and colleagues demonstrated that, among patients with IBS-D, response of diarrhea to a gluten-free diet was influenced by HLA-DQ2 positivity and the presence of IgG tissue transglutaminase antibody (TTG) in duodenal aspirates (9). Symptom response to gluten withdrawal occurred in 62% of patients positive for both HLA-DQ2 and IgG-TTG; in contrast, only 12% of patients negative for HLA-DQ2 and IgG-TTG responded, suggesting that symptom generation in this subset of patients is immune mediated.

Animal studies have suggested that HLA-DQ8 transgenic mice sensitized to gluten have increased contractile responses of intestinal smooth muscle to electrical field stimulation and to carbachol after gliadin exposure (10). This increased contractile activity may provide the basis for the development of dysmotility with gluten sensitivity. The role of HLA-DQ status in small bowel and colonic motor function in IBS patients has not been established.

Our study hypothesis was that IBS-D patients who are HLA-DQ2 or HLA-DQ8 positive have faster small bowel or colonic transit than HLA-DQ2 and HLA-DQ8 negative IBS-D patients. The aim of this study was to assess small bowel and colonic transit in IBS-D patients according to their HLA-DQ status. Understanding this relationship has the potential to optimize treatment of a subset of IBS-D patients.

METHODS

Patients and Study Design

Ninety-four patients with IBS-D were identified from a database of 700 people who were either healthy controls or had been diagnosed with a functional gastrointestinal disorder after clinical evaluation at Mayo Clinic and had been recruited for previous studies (1, 11). All participants were residing within 200 miles of Mayo Clinic, Rochester, MN.

These 94 patients fulfilled Rome II criteria for diagnosis of IBS-D (12). Thirty of these patients had not undergone transit measurements; thus, 64 patients had undergone measurements of gastric, small bowel, and colonic transit and were eligible for inclusion. During the transit studies, participants were allowed to continue stable doses of thyroid replacement, estrogen replacement, low-dose aspirin (81 mg per day), birth control pills or depot estrogen injection, and SSRI antidepressants, but not tricyclic agents.

Exclusion criteria included organic diseases that might explain the patients' symptoms, use of any medication for IBS or bowel dysfunction (within 7 days before the study and throughout the course of study), and any structural or metabolic diseases that affect the gastrointestinal system including diabetes. Patients who had participated in another clinical study within the prior 30 days were ineligible.

All individual studies had been approved by Mayo Clinic Institutional Review Board. Use of the database from which this analysis was conducted was also reviewed and approved by Mayo Clinic Institutional Review Board, and all participants had authorized use of their medical records and stored DNA for future research studies of gastrointestinal disease.

Evaluation for Celiac Disease

Patients' medical records were evaluated to assess if celiac disease had been excluded by either tissue transglutaminase (TTG) IgA and IgG or duodenal biopsies that were reviewed by an expert GI pathologist. For those in whom celiac disease had not been previously excluded, TTG IgA and IgG serology was performed on stored serum (N=33). All participants had provided prior authorization for future use of their serum for research. With IRB permission, some participants with insufficient stored serum were contacted by letter to provide additional serum (N=9).

Tissue transglutaminase IgA was measured by a commercially available enzyme immunoassay kit (BINDAZYME Human Anti-Tissue Transglutaminase IgA Kit, The Binding Site Limited, Birmingham B14 4ZB, UK). Tissue transglutaminase IgG was measured by a commercially available enzyme immunoassay kit (INOVA, San Diego, CA).

Measurement of Gastric Emptying, Small Bowel and Colonic Transit with Scintigraphy

Figure 1 shows the method used for transit studies and the specific time points of assessment for each of the measurements. Sixty-four patients had undergone measurements of gastric (N=50), small bowel (N=54), and colonic transit (N=64). Gastric emptying was measured over 4 hours after meal ingestion. One mCi ^{99m}Tc -sulfur colloid was added to two raw eggs during the scrambling, cooking process as in prior studies (12). The scrambled eggs were served on one slice of buttered bread with 240 ml of skim milk. The total calorie content of the meal was 296 kcal, and macronutrient composition was 32% protein, 35% fat, and 33% carbohydrate. Anterior and posterior gamma camera images were obtained immediately after radiolabeled meal ingestion, every 15 minutes for the first 2 hours, then every 30 minutes for the next 2 hours.

We assessed the colonic filling at 6 hours of the ^{99m}Tc egg meal. This serves as a valid surrogate for small bowel transit (13).

To evaluate colonic transit (14–16), ^{111}In adsorbed on activated charcoal particles was delivered to the colon by a methacrylate-coated, delayed-release capsule (15). All participants ingested the capsule containing ^{111}In charcoal at 6:00 a.m. after an overnight fast. When the capsule was demonstrated to have emptied from the stomach (with images obtained every 30 minutes while the patient was fasting), all participants received the radiolabeled egg meal at time 0. Two standardized meals were ingested at 4 hours (530 kcal

chicken meal) and 8 hours (750 kcal, roast beef sandwich, which includes two slices of bread). We obtained abdominal images every 30 minutes for the first 10 hours, with scans being increased to every 15 minutes for the first 2 hours after meals. A final scan was obtained at 48 hours. The performance characteristics of this test are summarized elsewhere (17).

A variable region of interest program was used to measure isotope counts in each region, and thereby derive a transit measurement, as in previous studies (14–16). Geometric means of counts in anterior and posterior gastric regions of interest were used (after correction for radioisotope decay) to estimate the proportion of ^{99m}Tc emptied from the stomach at each time point or filling the colon at 6 hours, and the proportion of ^{111}In in each colonic region at specified times.

Transit Data Analysis

Gastric emptying endpoints of analysis were $t_{1/2}$ for solids which were estimated by linear interpolation of the data at each time point. Small bowel transit time was assessed indirectly by the colonic filling at 6 hours. Overall colonic transit was summarized as the colonic geometric center (GC) at specified times. The GC is the weighted average of counts in the colonic regions (ascending, transverse, descending, rectosigmoid) and stool, respectively, 1 to 5. At any time, the proportion of counts in each colonic region is multiplied by its weighting factor as follows: (% ascending * 1 + % transverse * 2 + % descending * 3 + % rectosigmoid * 4 + % stool * 5)/100 = GC. Thus, a higher GC reflects a faster colonic transit.

Data on gastric emptying were available for 50, small bowel transit for 54, and colonic transit for all 64 participants. Analysis of associations was based on the available transit data.

HLA Genotyping

DNA was extracted from peripheral blood for HLA typing of DR and DQ alleles. High resolution typing of the specific DQ alpha and DQ beta alleles, based on a novel HLA genotyping method using six HLA tagging SNPs, was performed (18).

Statistical Analysis

The association of scintigraphic responses available (50 gastric emptying, 54 small bowel transit, and 64 colonic transit) with HLA-DQ2 and HLA-DQ8 status was assessed using analysis of covariance (ANCOVA), adjusting for BMI for small bowel and colonic transit, and adjusting for gender in the analysis of gastric emptying. The models also included an HLA-DQ2 by HLA-DQ8 cross-product term to check for potential differential associations for HLA-DQ2 or for HLA-DQ8, given the other genotype. The reported p-values for specific pair-wise comparisons are not adjusted for three tests (i.e., using the HLA-DQ2/HLA-DQ8 negative/negative group as the “control group”).

RESULTS

Patients and HLA-DQ Status

The mean age of the 64 patients with transit measurements was 40.8 ± 1.6 years, and 98.5% were female. Table 1 shows demographic data by the HLA-DQ status. There were no differences in age or BMI among the 4 groups. Sixty of the 64 patients had serological or duodenal histological exclusion of celiac disease. Serum and duodenal biopsy were not available for 4 participants.

Twenty-seven of the 64 patients were HLA-DQ2 positive; of these 27 patients, 16 were HLA-DQ subtype 2.2 and 11 were subtype 2.5. Among patients positive for HLA-DQ subtype 2.2, there were 1 homozygote and 15 heterozygotes. Of the 11 patients positive for HLA-DQ subtype 2.5, there were 3 homozygotes and 8 heterozygotes. The overall efficiency of the HLA SNP assay ranged from 90–100%. The mean allele frequency for each of the 6 SNPs studied in this cohort was not different from those reported in HapMap CEU (19).

Gastric Emptying of Solids

Fifty patients had t1/2 emptying time for solids. Figure 2 illustrates the gastric emptying data by HLA-DQ2 and HLA-DQ8 status independently. Gastric emptying t1/2 emptying time for solids was 126.3 ± 5.9 minutes in the HLA-DQ2+ subjects compared to 127.4 ± 7.8 minutes in the HLA DQ2- subjects, and 128.6 ± 7.7 minutes in the HLA-DQ8+ subjects compared to 126.1 ± 6.5 minutes in the HLA DQ8- subjects. HLA-DQ2 or HLA-DQ8 status did not significantly influence gastric emptying time.

Small Bowel Transit

Fifty-four patients had small bowel transit data expressed as percent of colonic filling at 6 hours. Percent colonic filling at 6 hours (CF6) was $56.0 \pm 7.4\%$ in HLA-DQ2+ patients compared to $50.3 \pm 4.3\%$ in HLA-DQ2- patients, and $62.3 \pm 7.2\%$ in HLA-DQ8+ patients compared to $48.4 \pm 4.6\%$ in HLA-DQ8- patients (Figure 3). Colonic filling at 6 hours in patients positive for HLA-DQ2 was numerically higher ($p=0.065$) compared to HLA-DQ2 negative. Independently, those positive for HLA-DQ8 had greater percent colonic filling at 6 hours (Figure 2) compared to HLA-DQ8 negative ($p=0.021$) patients. The ANCOVA indicated patients positive for both HLA-DQ2 and HLA-DQ8 had faster small bowel transit compared to those negative for HLA-DQ2 and HLA-DQ8 (Table 2, $p=0.013$).

Colonic Transit

All the patients had colonic transit measured as GC at 24 hours. There was no statistically significant association of colonic transit with patients' HLA-DQ2 or HLA-DQ8 status ($p>0.2$). However, the ANCOVA indicated that patient's positive for both HLA-DQ2 and HLA-DQ8 had numerically faster colonic transit compared to patients with both HLA-DQ2 and HLA-DQ8 negative ($p=0.38$) (Table 2). The analysis also suggested that HLA-DQ8 positivity had a greater impact on colonic transit if HLA-DQ2 was positive than if HLA-DQ2 was negative ($p=0.101$ for the HLA-DQ2 by HLA-DQ8 interaction term).

DISCUSSION

Our study shows that patients with IBS-D, positive for either HLA-DQ8 or both HLA-DQ2 and HLA-DQ8 genotypes that are associated with gluten sensitivity, have an accelerated small bowel transit time. Gastric emptying for solids was not influenced by HLA-DQ2 and HLA-DQ8 genotypes. This indicates that the greater colonic filling at 6 hours in HLA-DQ8 positive patients reflects accelerated small bowel transit. Colonic transit time was numerically, but not significantly, faster in patients positive for both HLA-DQ2 and HLA-DQ8 alleles.

A number of questions need to be addressed to appraise the data presented here: first, the performance characteristics of the positive result in the study, that is, the association of HLA-DQ status with small bowel transit. Colonic filling at 6 hours as a measurement of small bowel transit has been well validated. The reported intersubject coefficient of variation (COV) is approximately 30% and the intra-subject COV is 19% (17), supporting the robust nature of this test to evaluate small bowel transit. The inter- and intra-subject coefficients of

variation are similar for gastric emptying $t_{1/2}$, 30.4% and 14%, respectively (12). It is important to note that gastric emptying was not different among HLA-DQ groups. This is relevant because significant variations in gastric emptying times may impact the interpretation of colonic filling at 6 hours. Since gastric emptying times were not different by HLA-DQ status in this cohort, this does not influence the differences observed in colonic filling at 6 hours. We conclude that the association with HLA-DQ status is with small bowel transit in IBS-D patients.

Second, we needed to appraise the rate of HLA-DQ2 or HLA-DQ8 positivity in the study cohort relative to community controls. Thus, our observed prevalence of HLA-DQ positivity is not higher than that reported for the local Olmsted County population (20). As expected in a population of patients negative for celiac disease, the HLA-DQ 2.2 and 2.5 subtypes were rarely homozygotes.

The role of gluten sensitivity and HLA-DQ status in IBS-D is a matter of ongoing research. This study provides further information on the potential mechanisms that may explain symptom generation in this subset of patients. Ongoing prospective studies in our laboratory are addressing the potential effect of several weeks of gluten exclusion in the relief of symptoms and normalization of small bowel transit. Our hypothesis is that an abnormal immune response to gluten evokes chronic inflammation with secondary effects on small bowel motor function in a subset of IBS-D patients. An alternative hypothesis is that the effect of gluten may be related to the chemical nature of gluten itself, rather than induction of chronic inflammation. Prior studies in IBS patients have shown that 35% of IBS patients are HLA-DQ2 positive and, among these patients, 23% had increased intraepithelial lymphocytes and 30% had increased antibodies associated with celiac disease in duodenal aspirates, though there were no such antibodies in serum (8). The effect of chronic inflammation may be mediated by secreted cytokines. Thus, monocytes from HLA-DQ2 positive patients release 2 to 3 times more IL-8, a proinflammatory chemokine, than the HLA-DQ2 negative subjects (21). The HLA molecules, present in antigen-presenting cells, determine the MHC-peptide complex that leads to an immune response by binding antigenic peptides, such as gluten, and presents it to a T-cell with a subsequent immune response and intestinal inflammation. Under normal circumstances, gluten is not an antigen that produces an immune response. However, in genetically susceptible individuals, the HLA molecules are able to bind gluten and form an MHC-complex. The density and duration of this interaction determine the T-cell activation and immune response (22).

While several studies in the literature report morphological or functional evidence of inflammation or immune activation in mucosal biopsies from the colorectum as well as the small intestine of IBS patients (21), the cause of the inflammation is still incompletely elucidated and bacterial proteases are currently the most favored etiological factor. We believe that further studies are necessary to determine the cause in IBS patients of the low-grade inflammatory state as illustrated by changes in CD3+ cell counts, mast cell numbers and their proximity to nerves, and reduced serotonin reuptake transporter (SERT) (23–25). The latter has the potential to lead to higher tissue serotonin levels and the associated inflammatory effects of this transmitter (26). The contribution of these mucosal changes to the development of gastrointestinal symptoms in IBS-D patients is the subject of ongoing research.

One potential effect of the low-grade inflammatory state of IBS is abnormal mucosal barrier function through change in the cellular tight junctions, thereby increasing intestinal permeability. This has been previously described in first-degree relatives of patients with celiac disease (27). Increased intestinal permeability has been documented in vivo in patients with IBS-D and in vitro in mucosa from such patients (28–30). However, the cause

of the increased permeability is still the subject of research. A soluble factor derived from colonic mucosal biopsies of patients with IBS results in increased permeability of confluent intestinal cells (Caco-2) in culture (30). A potential role of dietary gluten may also be postulated. Thus, recent studies in celiac disease patients showed that patients with greater histological injury, as indicated by their Marsh classification, had abnormal intestinal permeability and zonulin levels, but had improvement in these factors after long-term gluten-free diet (31). However, to date, there are no studies on intestinal permeability in susceptible IBS-D patients with gluten sensitivity or by HLA-DQ status.

The observation of TTG antibodies in intestinal fluid raises the interesting hypothesis that patients with certain HLA status may be susceptible to the production of TTG antibodies locally in the intestine and that this may injure the mucosa, increasing permeability and setting up the cascade of events that ultimately results in accelerated small bowel transit. This is analogous to the observation of Verdu et al. in an animal model where HLA-DQ8 predisposed to the development of motor abnormalities on exposure to gluten (10). Thus, although human studies are lacking, previous animal studies suggested that HLA-DQ8 transgenic mice that were sensitized to gluten had increased contractile responses in intestinal smooth muscle to electrical field stimulation and carbachol after gliadin exposure (10).

The pitfalls of this study include its descriptive and retrospective nature, the sample size of participants with transit measurements, and the lack of data on the dietary habits of the patients. Clearly, our study should be regarded as hypothesis-generating, and confirmation in replication studies is essential. In order to assess the study's power, post-hoc estimates of the degree of association that could have been detected with 80% power (2-sided alpha level=0.05) and expressed as percent differences between groups defined by HLA DQ2 status (positive vs. negative) and, separately, DQ8 status (positive vs. negative) are given in Table 3 for each transit measurement. Note that the effect size (difference) in each case is within the range that would be clinically relevant for that endpoint. Therefore, we do not perceive that the sample sizes resulted in a type II error, specifically for those transit profiles that were not significant, that is, gastric emptying and colonic transit.

With regard to dietary habits, it is unknown if any patient in this cohort had been following a gluten-free diet, in which potentially susceptible subjects would be devoid of symptoms at the time of evaluation. The meals administered in this study did contain bread and gluten. While the total amount of gluten administered in the test meals was relatively small, we are unable to determine whether the contained gluten actually influenced the transit profile. Serum or tissue was available to exclude celiac disease in 93% of our cohort, and they had normal duodenal biopsies or negative serum TTG IgA and IgG. The latter has previously been shown to have strong negative predictive value for celiac disease (32). The reported prevalence of celiac disease in previously diagnosed IBS-D patients is less than 5% (33).

Conclusion

In summary, IBS-D patients positive for HLA-DQ8, or both HLA-DQ2 and HLA-DQ8, have faster small bowel transit. Our data are consistent with the hypothesis that HLA-DQ subtype is an immunogenetic predisposing factor that, upon gluten exposure, leads to acceleration of small bowel transit that is clinically translated in generation of symptoms in IBS patients who do not have celiac disease. The mechanism of the accelerated transit and the potential impact of gluten exposure to small bowel function deserve further investigation.

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Figure 1.
Transit studies and the time points of assessment

Figure 2.
Effect of HLA-DQ2 or HLA-DQ8 on gastric emptying of solids

Figure 3.
Effect of HLA-DQ2 or HLA-DQ8 on small bowel transit

Table 1

Demographics of IBS-D Cohort

	DQ2 -, DQ8 -	DQ2 +, DQ8 -	DQ2 -, DQ8 +	DQ2 +, DQ8 +
N	25	23	12	4
Age, years	41.2 ± 1.9	40.4 ± 3.3	41.9 ± 3.6	37.5 ± 5.5
Gender female, N (%)	23 (92)	22 (96)	12 (100)	4 (100)
BMI, kg/m ²	28.2 ± 1.3	28.6 ± 1.1	29.0 ± 1.7	30.9 ± 4.6
TTG negative, N (%)	23 (92)	23 (100)	11(92)	3(75)
TTG unknown, N (%)	2 (7)	0 (0)	1(8)	1(25)

Note: All data shown are mean ± SEM, unless indicated as N (%).

Table 2

Small Bowel and Colonic Transit Data in IBS-D

HLA genotype	N	Colonic Filling at 6h, %	N	GC 24
DQ2 -, DQ8 -	20	48.0 ± 5.2	25	3.3 ± 0.3
DQ2 +, DQ8 -	18	48.8 ± 7.9	23	3.0 ± 0.2
DQ2 -, DQ8 +	12	54.1 ± 8.0	12	2.9 ± 0.3
DQ2 +, DQ8 +	4	88.8 ± 8.7*	4	3.9 ± 0.6

*
p = 0.013

Table 3
Statistical Power

Post-hoc estimates of main effects size demonstrable with 80% power ($\alpha=0.05$) based on number of participants studied for each regional transit: DQ8 Status				
Transit endpoint	CV(%)	DQ8(+) N	DQ8(-) N	EFFECT SIZE (%) [†]
GE T ½	28	15	35	25
CF at 6hrs	55	15	38	48.5
GC at 24hrs	36	16	38	31
GC at 48hrs	24	15	40	21

Post-hoc estimates of main effects size demonstrable with 80% power ($\alpha=0.05$) based on number of participants studied for each regional transit: DQ2 Status				
Transit endpoint	CV(%)	DQ2(+) N	DQ2(-) N	EFFECT SIZE (%) [†]
GE T ½	28	21	29	23.1
CF at 6hrs	55	21	33	44.2
GC at 24hrs	36	26	38	26.3
GC at 48hrs	24	23	32	18.9

[†] EFFECT SIZE IS 100*(Difference in Means)/(Mean overall subjects)