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Factors associated with villus atrophy in symptomatic coeliac disease patients on a gluten-free diet

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SUMMARY

Background
Duodenal injury persists in some coeliac disease patients despite gluten-free diet, and is associated with adverse outcomes.

Aim
To determine the prevalence and clinical risk factors for persistent villus atrophy among symptomatic coeliac disease patients.

Methods
A nested cross-sectional analysis was performed on coeliac disease patients with self-reported moderate or severe symptoms while following a gluten-free diet, who underwent protocol-mandated duodenal biopsy upon enrolment in the CeliAction clinical trial. Demographic factors, symptom type, medication use, and serology were examined to determine predictors of persistent villus atrophy.

Results
Of 1345 symptomatic patients, 511 (38%, 95% CI, 35–41%) were found to have active coeliac disease with persistent villus atrophy, defined as average villus height to crypt depth ratio ≤2.0. On multivariable analysis, older age (OR, 5.1 for ≥70 vs. 18–29 years, 95% CI, 2.5–10.4) was a risk factor while longer duration on gluten-free diet was protective (OR, 0.37, 95% CI, 0.24–0.55 for 4–5.9 vs. 1–1.9 years). Villus atrophy was associated with use of proton-pump inhibitors (PPIs; OR, 1.6, 95% CI, 1.1–2.3), non-steroidal anti-inflammatory drugs (NSAIDs; OR, 1.64, 95% CI, 1.2–2.2), and selective serotonin reuptake inhibitors (SSRIs; OR, 1.74, 95% CI, 1.2–2.5). Symptoms were not associated with villus atrophy after adjusting for covariates.

Conclusions
A majority of symptomatic coeliac disease patients did not have active disease on follow-up histology. Symptoms were poorly predictive of persistent mucosal injury. The impact of NSAIDs, PPIs, and SSRIs on mucosal healing in coeliac disease warrants further study.

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INTRODUCTION

In susceptible persons, exposure to gluten-containing foods triggers autoimmune injury to the lining of the small bowel, resulting in the characteristic lesion of coeliac disease.[1] Duodenal mucosal biopsy demonstrates intraepithelial lymphocytosis, crypt hyperplasia and villus atrophy. Strict avoidance of gluten is the mainstay of treatment and can reverse the clinical and histologic features of the disease.

A significant proportion of coeliac disease patients do not heal their small bowel mucosa despite attempts at gluten avoidance. The prevalence of persistent villus atrophy upon follow-up duodenal biopsy is variably reported, and is influenced by characteristics of the study sample as well as design and duration of follow-up.[2] The largest population-based study, based on 7648 coeliac disease patients in Sweden, found persistent villus atrophy in 43% of patients undergoing follow-up biopsy. Persistent villus atrophy may be found even in asymptomatic patients with negative coeliac serologies, and is thus distinct from refractory coeliac disease, the definition of which includes continued symptoms and signs of malabsorption in addition to persistent villus atrophy.[3–7]

Persistent villus atrophy has been associated with serious sequelae, including lymphoproliferative malignancy and osteoporotic fractures.[8, 9] While patients with coeliac disease are at slightly increased risk of early death compared to the general population, evidence linking persistent villus atrophy to increased mortality is lacking.[10, 11] Nevertheless, given its associated morbidity, coeliac disease management is increasingly targeting mucosal healing, with follow-up duodenal biopsy to confirm histologic remission becoming more frequent, especially in patients with ongoing symptoms.

Factors known to be associated with persistent villus atrophy include older age at diagnosis/follow-up, male sex, and low educational attainment.[2] Adults are significantly less likely to normalise their duodenal histology than children.[12] The presence of gastrointestinal symptoms while on a gluten-free diet has been evaluated in one study, and found to be associated with persistent villus atrophy.[13] The relevance of other types of symptoms on risk of persistent villus atrophy has not, however, been studied. Drug-induced enteropathy is well recognised and has been linked to use of angiotensin-receptor blockers, non-steroidal anti-inflammatory drugs (NSAIDs), and possibly proton-pump inhibitors (PPIs), however the impact of use of these medications on healing of villus atrophy in coeliac disease is not known.[14–17]

We therefore sought to determine associations between clinical factors, including symptom type, medication use, and laboratory findings, and the risk of persistent villus atrophy among symptomatic coeliac disease patients following a gluten-free diet undergoing protocol-mandated duodenal biopsy upon entering a clinical trial.

MATERIALS AND METHODS

Study population

We conducted a nested cross-sectional study on a pre-randomisation cohort of 1345 patients from CeliAction (clinicaltrials.gov identifier NCT01917630), a multicenter, randomised clinical trial evaluating the effect of latiglutenase (formerly ALV003, Alvine Pharmaceuticals, San Carlos, CA, USA), a gluten-specific enzyme therapeutic agent.[18] The trial recruited adults with coeliac disease from North America, the United Kingdom, Ireland, Norway and Finland. In order to be screened, patients had to have been diagnosed with coeliac disease by a physician and to have persistent symptoms despite following a gluten-free diet for at least 1 year. Persistent symptoms were defined as self-reporting at least one moderate or severe-intensity gastrointestinal symptom (e.g. abdominal pain, bloating, diarrhoea, constipation or nausea) within the 28 days prior to screening. Patients who met the screening criteria were administered the Celiac Disease Symptom Diary daily for 28 days via a telephonic interactive voice response system. This coeliac disease-specific patient reported outcome measure records the frequency and severity of diarrhoea, constipation, abdominal pain, bloating, nausea and tiredness. Subjects who reported at least one moderate or severe symptom from the instrument during days 15 to 28 following screening were included in the duodenal biopsy cohort who underwent oesophagogastroduodenoscopy (EGD). The present analysis comprises all 1345 patients from CeliAction who underwent EGD with duodenal biopsy.

Duodenal biopsies

EGD was performed by experienced endoscopists following standard procedures for sedation and endoscopy. Mucosal biopsies were obtained with a cold forceps from the second portion of the duodenum. Four specimens were obtained using a one-bite-per-pass technique as per the trial protocol. All biopsies were individually fixed in formalin, centrally processed to ensure uniform specimen processing and orientation, stained with haematoxylin-

eosin and evaluated by experienced gastrointestinal pathologists unaware of serology or clinical status. Villus heights and crypt depths were measured in multiple locations. The average villus height to crypt depth ratio (VH:CD) was calculated from 5 to 12 well-oriented villus-crypt units for each patient.

Persistent villus atrophy, the primary outcome of this study, was defined as VH:CD of ≤ 2.0. As a secondary outcome, we defined severe persistent villus atrophy as VH:CD ≤ 1.0.

Symptoms and medication use

Symptoms were obtained from the initial screening interview and the 2-week run-in period following screening. Symptoms of any intensity were coded as present and analysed as binary variables. A snapshot of all medications taken by patients at the time of the screening interview was recorded. Drugs were grouped by class (e.g. NSAID, PPI) and analysed as binary variables.

Serologic data

Blood obtained at the time of screening was analysed using common assays for all participants. For coeliac serologies, an enzyme-linked immunosorbent assay from INOVA Diagnostics (San Diego, California, United States) was used to measure tissue transglutaminase IgA (TTG), and deamidated gliadin peptide (DGP) IgA and IgG levels. Liver enzymes, comprising aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP), were assayed using standard techniques. Each serologic test was compared with its reference interval and analysed as a binary variable (i.e. elevated or not). As a supplementary analysis, the multiple of the upper limit of normal reference range for each serological test was analysed as a continuous variable. A subset of patients with persistent villus atrophy was tested for human leucocyte antigen genotype.

Statistical analysis

Patient demographics, symptoms and medication use were summarised using means, medians and proportions as appropriate. The primary outcome (persistent villus atrophy, VH:CD ≤ 2.0) was compared between those who did and did not report each symptom, between users and non-users of each class of medication, and among those with elevated and normal serologic tests. The secondary outcome (severe persistent villus atrophy, VH:CD ≤ 1.0) was compared among medication users and non-users. *P*-values for categorical comparisons were calculated using Pearson's chi-squared test. We performed multiple logistic regressions to identify independent predictors of persistent villus atrophy. All covariates found to be statistically significant on univariable analysis were included in the multivariable model. Supplementary analyses included logistic regression modelling severe persistent villus atrophy, and linear regression modelling VH:CD as a continuous outcome. All statistical analyses were performed using Stata Release 13 (StataCorp; College Station, TX). This study was approved by the institutional review board of Columbia University Medical Center. CeliAction was a registered clinical trial (https://clinicaltrials.gov/ct2/show/NCT01917630) and was conducted in accordance with the ICH guideline E6 Good Clinical Practice, the Declaration of Helsinki, European Union Directives and with applicable local regulations governing clinical trials. All patients gave written informed consent. All authors had access to the data, reviewed and approved the final manuscript.

RESULTS

Patient characteristics

A majority of 1,345 patients were female (81%) and white (98%), with a mean age of 46 years (Table 1). The median duration on a gluten-free diet was 4 years, with one-fifth of the sample having commenced the diet within 2 years of screening. The primary outcome of persistent villus atrophy was found in 511 patients (38%, 95% CI, 35–41%), which includes 100 patients (7%, 95% CI, 6–9%) who also met the criteria for severe persistent villus atrophy.

Males were more likely than females to have persistent villus atrophy (45% vs. 36%, $P = 0.011$) on univariable analysis (Table 2). Older age was monotonically associated with risk of persistent villus atrophy, ranging from 25% among 18–29 year olds up to 62% among those aged 70 or older. Longer duration on gluten-free diet was protective with regard to persistent villus atrophy, with a plateau after 4 years (Figure 1). Those on a gluten-free diet for less than 2 years were at highest risk (52%) of the primary outcome. Among the subset ($n = 457$) of patients with persistent villus atrophy who underwent genetic testing for human leucocyte antigens, 359 (79%) were found to be DQ2 positive, 48 (11%) were DQ8 positive, and 38 (8%) were positive for both DQ2 and DQ8.

Table 1 | Characteristics of patients with coeliac disease and persistent symptoms (n = 1345) who underwent duodenal biopsy

	n (%)
N	1345
Female sex	1089 (81.0)
Age in years, mean (s.d.)	46.3 (14.2)
18–29	201 (14.9)
30–39	244 (18.1)
40–49	301 (22.4)
50–59	315 (23.4)
60–69	219 (16.3)
≥70	65 (4.8)
Race	
White	1312 (97.6)
Native American	11 (0.8)
Black	4 (0.3)
Asian	4 (0.3)
Other	11 (0.8)
Measurements	
Height, mean (s.d.) in cm	
Females	165 (10.9)
Males	178 (10.1)
BMI, mean (s.d.)	27.0 (5.9)
<20	81 (6.1)
20–24.9	499 (37.3)
25–29.9	410 (30.7)
≥30	347 (26.0)
Duration of gluten-free diet in years	
Median (IQR)	4.0 (1.9–7.4)
1–1.9	293 (21.8)
2–3.9	321 (23.9)
4–5.9	252 (18.7)
6–7.9	182 (13.5)
≥8	297 (22.1)
Villus atrophy	
Persistent (VH:CD≤2)	511 (38.0)
Severe persistent (VH:CD≤1)	100 (7.4)
Symptoms	
Bloating	1167 (86.8)
Abdominal pain	1134 (84.3)
Tiredness	1129 (83.9)
Diarrhoea	1018 (75.7)
Nausea	690 (51.3)
Constipation	684 (50.9)
Depression/anxiety	431 (32.0)
Heartburn	327 (24.3)
Headache	320 (23.8)
Anaemia	262 (19.5)
Hypertension	179 (13.3)
Dermatitis herpetiformis	81 (6.6)
Current medication use	
NSAID	362 (27.3)
PPI	349 (26.4)
SSRI	209 (15.8)
Statin	127 (9.6)
Histamine-2 receptor antagonist	60 (4.5)
Angiotensin receptor antagonist	18 (1.4)
Elevated coeliac antibodies	
DGP IgA	235 (17.5)
DGP IgG	205 (15.3)
TTG IgA	197 (14.7)
At least one of the above	303 (22.5)
Abnormal laboratory tests	
AST	125 (9.4)
ALT	187 (14.0)
GGT	96 (7.2)
Alkaline phosphatase	147 (11.0)

Table 2 | Demographics and persistent villus atrophy (VH:CD ≤2)

	Proportion with villus atrophy (VH:CD ≤2)	*P*
Sex		0.011
Male	115/256 (44.9)	
Female	396/1089 (36.4)	
Age		<0.001
18–29	50/201 (24.9)	
30–39	72/244 (29.5)	
40–49	108/301 (35.9)	
50–59	141/315 (44.8)	
60–69	100/219 (45.7)	
≥70	40/65 (61.5)	
Race		0.528*
White	495/1312 (37.7)	
Native American	5/11 (45.5)	
Black	1/4 (25.0)	
Asian	2/4 (50.0)	
Other	8/14 (57.1)	
BMI		0.014
<20	28/81 (34.6)	
20–24.9	165/499 (33.1)	
25–29.9	177/410 (43.2)	
≥30	138/347 (39.8)	
Duration on gluten-free diet in years		<0.001
1–1.9	151/293 (51.5)	
2–3.9	108/321 (33.6)	
4–5.9	82/252 (32.5)	
6–7.9	63/182 (34.6)	
≥8	107/297 (36.0)	

* Fishers exact test; remainder are Pearson chi-squared tests.

Symptoms and persistent villus atrophy

All patients in this study reported at least one symptom in order to meet the inclusion criteria. Individual symptoms were reported at high rates (Table 1), most notably

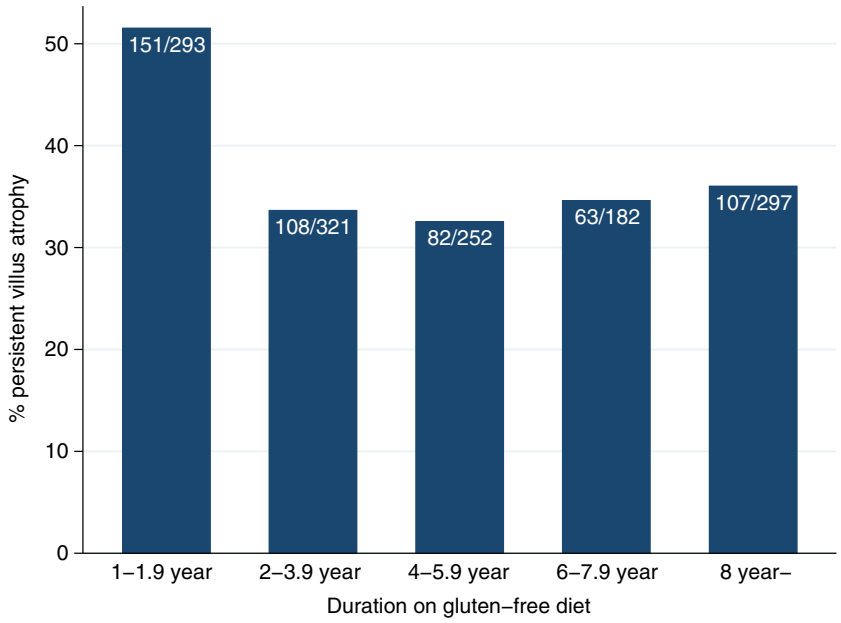

Figure 1 | Prevalence of villus atrophy among 1345 coeliac disease patients with persistent symptoms, grouped by duration on a gluten-free diet.

bloating (87%), abdominal pain (84%), tiredness (84%) and diarrhoea (76%). Patients who reported hypertension, heartburn or anaemia were slightly more likely to have persistent villus atrophy than those who did not report these disorders (Table 3). The presence of certain symptoms was inversely associated with risk of persistent villus atrophy, including abdominal pain (36% if abdominal pain present vs. 48% if absent, $P = 0.001$), nausea (35% vs. 42%, $P = 0.009$) and bloating (37% vs. 45%, $P = 0.04$).

Medication use and persistent villus atrophy

Non-steroidal anti-inflammatory drug and PPI use was highly prevalent, with over a quarter of the sample listing medications within each of these classes (Table 1). The risk of persistent villus atrophy was increased among users of PPIs compared to non-users (48% vs. 35%, $P < 0.001$), as well as for users of NSAIDs, selective serotonin reuptake inhibitors (SSRIs) and statins (Table 3). Only PPI use was associated with increased risk of severe persistent villus atrophy (Table S1).

Serological markers and persistent villus atrophy

At least one coeliac antibody was abnormal in 303 (23%) patients in the sample (Table 1). The DGP IgG antibody was highly associated with persistent villus atrophy (81% vs. 30%, $P < 0.001$), as were DGP IgA and TTG IgA, and to a lesser extent, serum transaminases (Table 4).

On multivariable analysis (Table 5), older age and shorter duration on gluten-free diet predicted persistent villus atrophy. Significant associations remained with use of PPIs, NSAIDs and SSRIs. The DGP IgG was highly predictive of persistent villus atrophy (OR, 6.6, 95% CI, 4.3–10.3), as was TTG IgA. Sex, body mass index (BMI) and notably symptom type were not associated with persistent villus atrophy after adjusting for other covariates. Predictors of severe villus atrophy (Table S2) included age ≥70, elevated coeliac serology, and an inverse association with nausea (OR, 0.55, 95% CI, 0.32–0.95). Both abdominal pain and nausea were also associated with higher VH:CD values (Table S3), again suggesting an inverse association with risk of persistent villus atrophy.

DISCUSSION

In this study of 1345 symptomatic coeliac disease patients following a gluten-free diet who volunteered to participate in a study of a recombinant, gluten-specific enzyme preparation for the treatment of symptomatic coeliac disease, 38% had persistent villus atrophy. We analysed medication use, symptom characteristics and serology to identify factors associated with persistence of villus atrophy despite gluten-free diet. Notably, we found the use of medications within three classes – PPIs, NSAIDs and SSRIs – independently predicted persistent villus atrophy: a novel finding. While patients frequently reported abdominal pain, nausea and bloating, those who did were in fact *less* likely to have abnormal biopsy findings, reflecting the multiple etiologies that can account for abdominal symptoms in these patients. Demographic factors including age and duration of the gluten-free diet were strongly associated with impaired

Table 3 | Univariate analysis of symptoms and medication use with persistent villus atrophy (VH:CD ≤2)

	Proportion with villus atrophy (VH:CD ≤2)		*P*
Symptoms	Present	Absent	
Bloating	431/1167 (36.9)	80/178 (44.9)	0.04
Abdominal pain	410/1134 (36.2)	101/211 (47.9)	0.001
Tiredness	421/1129 (37.3)	90/216 (41.7)	0.23
Diarrhoea	375/1018 (36.8)	136/327 (41.6)	0.12
Nausea	239/690 (34.6)	272/655 (41.5)	0.009
Constipation	263/684 (38.5)	248/661 (37.5)	0.73
Depression/anxiety	173/431 (40.1)	338/914 (37.0)	0.27
Heartburn	140/326 (42.8)	371/1018 (36.4)	0.04
Headache	112/320 (35.0)	399/1025 (38.9)	0.21
Anaemia	114/262 (43.5)	397/1083 (36.7)	0.04
Hypertension	80/179 (44.7)	431/1166 (37.0)	0.05
Dermatitis herpetiformis	31/89 (34.8)	480/1256 (38.2)	0.52
Medications	Users	Non-users	
NSAID	168/362 (46.4)	339/962 (35.2)	<0.001
PPI	167/349 (47.9)	340/975 (34.9)	<0.001
SSRI	98/209 (46.9)	409/1115 (36.7)	0.005
Statin	67/127 (52.8)	440/1197 (36.8)	<0.001
Histamine-2 receptor antagonist	29/60 (48.3)	478/1264 (37.8)	0.102
Angiotensin receptor antagonist	8/18 (44.4)	499/1306 (38.2)	0.589

Table 4 | Laboratory tests and persistent villus atrophy (VH:CD ≤2)

	Proportion with villus atrophy (VH:CD ≤2)		
	Elevated serum level	Normal serum level	*P*
Coeliac antibodies			
DGP IgA	156/235 (66.4)	353/1107 (31.9)	<0.001
DGP IgG	166/205 (81.0)	343/1137 (30.2)	<0.001
TTG IgA	136/197 (69.0)	373/1145 (32.6)	<0.001
Any coeliac Ab+	203/303 (67.0)	308/1042 (30.0)	<0.001
Liver enzymes			
AST	63/125 (50.4)	444/1210 (36.7)	0.003
ALT	88/187 (47.1)	419/1149 (36.5)	0.006
GGT	43/96 (44.8)	464/1239 (37.5)	0.153
Alkaline phosphatase	62/147 (42.2)	445/1188 (37.5)	0.266

mucosal healing, as has been shown previously. As expected, coeliac disease antibodies were associated with villus atrophy; however, this analysis found the DGP IgG antibody to be superior to both DGP IgA and TTG IgA in indicating the presence of a persistent histologic lesion.

To our knowledge, this is the largest study of follow-up duodenal biopsy in coeliac disease using a protocol-specified collection of symptoms, laboratory values and standardised biopsy collection and quantitative interpretation. It is also the first study to our knowledge to examine the relationship between medication use and mucosal healing in coeliac disease. Several classes of medications have been implicated as primary factors that can cause small-bowel injury in noncoeliac individuals. NSAID-induced enteropathy is well described and results in villus denudation, erosions, and ulcers, in part due to inhibition of cytoprotective enterocyte factors.[19] Olmesartan can cause a severe sprue-like enteropathy that mimics coeliac disease histologically, and which improves following medication cessation.[20] There are case reports of similar occurrences with other members of the

Table 5 | Multiple logistic regression of factors associated with persistent villus atrophy (VH:CD ≤2)

Covariate	OR	95% CI	*P*
Male sex	1.30	0.93–1.83	0.129
Age			
18–29 (ref)	1		
30–39	1.35	0.83–2.20	0.220
40–49	1.85	1.16–2.97	0.011
50–59	2.51	1.55–4.07	<0.001
60–69	2.40	1.43–4.04	0.001
≥70	5.08	2.48–10.42	<0.001
BMI			
<20	1.37	0.78–2.40	0.275
20–24.9 (ref)	1		
25–29.9	1.15	0.83–1.58	0.398
≥30	0.86	0.61–1.23	0.406
Duration on gluten-free diet in years			
1–1.9 (ref)	1		
2–3.9	0.47	0.32–0.68	<0.001
4–5.9	0.37	0.24–0.55	<0.001
6–7.9	0.41	0.26–0.65	<0.001
≥8	0.40	0.27–0.60	<0.001
Symptoms			
Bloating	0.80	0.54–1.19	0.268
Abdominal pain	0.83	0.57–1.19	0.311
Nausea	0.79	0.60–1.03	0.083
Heartburn	0.89	0.62–1.26	0.497
Anaemia	1.22	0.88–1.68	0.236
Hypertension	0.81	0.54–1.20	0.300
Medication use			
PPI	1.60	1.13–2.27	0.008
SSRI	1.74	1.23–2.47	0.002
NSAID	1.64	1.24–2.18	0.001
Statin	1.21	0.77–1.89	0.404
Histamine-2 receptor antagonist	1.14	0.61–2.12	0.218
Elevated laboratory tests			
DGP IgA	1.30	0.86–1.98	0.218
DGP IgG	6.57	4.28–10.32	<0.001
TTG IgA	1.98	1.27–3.09	0.003
ALT	1.47	1.01–2.13	0.043

angiotensin receptor antagonist class; however, this has not been demonstrated in larger studies.[14, 21] In our study, angiotensin receptor antagonist use was not associated with impaired mucosal healing in this study; however, the overall prevalence of use was too low to detect an effect size of a similar magnitude to that seen for NSAIDs and PPIs. Statins were highly associated with persistent villus atrophy on univariate analysis, but this was likely due to its association with older age; after adjusting for age in the multivariable model there was no independent effect. The use of SSRIs – sertraline in particular – has been associated with secretory diarrhoea and microscopic colitis, but not previously with enteropathy and it is unclear by what mechanism they may be contributing to the persistent villus atrophy observed among users in this study.[22]

PPI sequelae have been extensively studied. However, an association with persistent villus atrophy in coeliac disease has not previously been reported. PPI use has been associated at a population level with subsequent development of coeliac disease.[23] Among patients newly diagnosed with coeliac disease, those on acid suppression medications have been reported to be more often seronegative with regard to coeliac disease autoantibodies than non-users.[24] The mechanism by which PPIs might influence mucosal healing is not clear; however, antisecretory agents may modulate the response to ingested antigens. PPIs have been shown to impair mucosal barrier function in the upper gastrointestinal tract.[25, 26] Increased rates of food-induced allergic responses have been demonstrated among mice and humans on acid suppression compared to controls.[27, 28] Gluten is at least partially susceptible to peptic degradation, although the extent to which medication-induced hypochlorhydria interferes with this process and the immunogenicity of the resultant products is not known.[23] Evidence is also mounting that PPIs can interact synergistically with NSAIDs to exacerbate small intestinal mucosal injury, possibly by inducing intestinal dysbiosis.[16, 29] It seems reasonable that through one or a combination of these mechanisms, that there may be a plausible basis for impaired mucosal healing in coeliac disease patients on PPIs.

The diagnostic value of symptoms as predictors of persistent villus atrophy has not been well established, and our results do not support specific symptoms as indicative of mucosal disruption. Persistent villus atrophy certainly can occur in the absence of any identifiable symptoms. One study compared 18 patients with persistent villus atrophy to 18 coeliac disease controls who achieved mucosal healing, and administered validated symptom assessment instruments to both groups.[6] Gastrointestinal and psychological symptom scores were no different between cases and controls. Among a cohort of 263 patients who underwent follow-up biopsy after 1 year on a gluten-free diet, patients who reported malabsorptive symptoms at the time of initial diagnosis were more likely to have persistent mucosal injury, however gastrointestinal symptoms at the time of follow-up did not predict histology.[30] Another study compared 42 symptomatic and 27 asymptomatic coeliac disease patients, all on the gluten-free diet, who underwent follow-up upper endoscopy.[13] Symptomatic patients were

far more likely than asymptomatic patients to have persistent villus atrophy (85% vs. 33%, $P < 0.0001$) on duodenal biopsy. Symptoms considered "typical" of coeliac disease (diarrhoea, anaemia, and weight loss) as well as heartburn, diffuse abdominal pain, and constipation were all found to be associated with persistent villus atrophy. We found no independent association between any symptoms and villus atrophy, contrasting with this study for several reasons. First, our study population was pre-selected to be symptomatic in order to enrol in a clinical trial. As such, comparison between symptomatic and entirely asymptomatic individuals is not possible. Second, the overall symptom frequencies are very high in our sample, and it is plausible that patients may have been particularly motivated to report symptoms as persistent symptoms were a criterion for inclusion in a trial in which they could potentially receive a novel therapeutic agent. Symptoms reported at a lower-than-usual threshold would bias these results towards the null. Finally, each of these studies used different symptom assessment instruments, limiting comparability.

The finding that the DGP antibody is useful for follow-up of mucosal healing is consistent with prior reports. The DGP IgA has previously been found to be most accurate for assessing dietary compliance soon after gluten-free diet initiation, and for predicting persistent villus atrophy in a comparison of several different classes of IgA coeliac antibodies.[31–33] The IgG DGP antibody has traditionally been used as a diagnostic modality in IgA-deficient patients. Our findings suggest that when positive, the DGP IgG serology is more predictive of persistent villus atrophy than other serologies.

Strengths of the present study include a high-quality, complete data set that is a product of a cross-sectional design nested within a prospective clinical trial. The study includes a large sample drawn from multiple centers throughout North America and Europe, improving generalisability. The design afforded contemporaneous collection of exposure and outcome data, which is particularly relevant when assessing symptom-outcome associations and limits recall bias. All pathology was centrally processed and reported quantitatively according to standardised protocols to assure validity and consistency. Similarly, serologic assays were standardised across all patients. These measures to decrease variance are rarely feasible in observational studies of this size.

Our study also has a number of limitations. Residual confounding is likely to persist despite adjusting for major covariates. Particularly with medication use, protopathic bias and reverse causation may underlie the relationships between exposures and the outcome of interest. In this study, patients with persistent villus atrophy may plausibly have experienced low-grade symptoms, which triggered more frequent physician visits and more prescriptions for PPIs, NSAIDs, and SSRIs. However, this is unlikely to be the case, since symptoms themselves were not found to be associated with persistent villus atrophy, suggesting that other mechanisms may be contributing. Granular information regarding the duration, dose, and frequency of medication use was not available from the screening interview. Neither dietary adherence data nor dietitian use were collected, hence it is not possible to assess if dietary noncompliance may have contributed to the link between the identified risk factors and persistent villus atrophy.

Endoscopic biopsy in patients with coeliac disease on a gluten-free diet is frequently performed to document mucosal healing, mainly because of the prognostic significance of the finding of persistent villus atrophy, and both physicians and patients are aware that healing of villus atrophy is the goal of therapy. In addition, endoscopy may be performed to determine the cause of persistent symptoms despite attempted adherence to the gluten-free diet. The presence of symptoms does not accurately predict the presence of persistent villus atrophy, with over 60% of these symptomatic patients lacking villus atrophy on biopsy. Thus, the majority of the patients with symptoms who enrolled in this study were likely to have had another cause of their symptoms, apart from active coeliac disease. Causes of persistent symptoms such as abdominal pain and bloating (the most common symptoms reported in these patients) may include sugar malabsorption due to lactose or fructose intolerance, small intestinal bacterial overgrowth, as well as functional disorders such as dysmotility and irritable bowel syndrome.[34, 35]

In summary, mucosal healing is far from universal in coeliac disease despite the gluten-free diet. In this study, we identified several novel risk factors for persistent villus atrophy. In particular, use of NSAIDs, PPIs and SSRIs appears to be associated with impaired mucosal healing. We also found that the DGP IgG serology appears to be the most reliable marker for persistent villus atrophy. Symptoms appear to be a poor predictor of histologic damage on follow-up in coeliac disease. Further prospective and mechanistic studies are needed to explore the mechanisms by which these identified associations, particularly medications, might be causal.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Medication use and severe persistent villus atrophy (VH:CD ≤1).

Table S2. Multiple logistic regression of factors associated with severe persistent villus atrophy (VH:CD ≤1).

Table S3. Linear regression modelling villus height to crypt depth ratio (VH:CD) as a continuous outcome.

AUTHORSHIP

Guarantor of the Article: Dr. Adelman.

Authors Contributions: SM, PHRG, DA, BL, JAM, TTW, CPK, MM, VC, MT: study concept and design; DA, JAM, TTW, CPK, MM, VC, MT: acquisition of data; SM, PHRG, DA, BL: analysis and interpretation of data; SM: drafting of the manuscript; SM, PHRG, DA, BL, JAM, TTW, CPK, MM: critical revision of the manuscript for important intellectual content; SM, BL: statistical analysis; BL: study supervision.

All authors approve the final manuscript submitted and they approve the authorship list.

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