Celiac disease serology and gut microbiome following proton pump inhibitor treatment

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Abstract

Background: Celiac disease is an autoimmune enteropathy characterized by an aberrant immune response to ingested gluten in genetically predisposed individuals. Studies have pointed to a rising prevalence of celiac disease in recent decades. Changes in diet and use of medication that may impact the gut microbiome have been suggested as potential contributors. Exposure to proton pump inhibitors (PPIs) was recently found to be associated with an increased risk for subsequent diagnosis of celiac disease. We aimed to investigate potential mechanisms for this link by examining the relationship between PPI use and gluten-related immune responses in the context of changes in gut microbiome.

Methods: We performed a post hoc analysis of blood and fecal samples from a recent randomized trial in order to assess the potential association between PPI use and development of celiac disease serology in conjunction with alterations in gastrointestinal microbial composition. The study included 12 healthy participants who were administered a PPI (Omeprazole; 40 mg twice daily) for 4 or 8 weeks.

Results: The analysis did not reveal an overall significant change in levels of serologic markers of celiac disease for the study cohort in response to PPI treatment. However, one individual developed a marked increase in the celiac disease-specific autoantibody response to transglutaminase 2 in conjunction with enhanced immune reactivity to gluten during the trial. Genotyping revealed positivity for the celiac disease-associated HLA-DQ2 and -DQ8 alleles. Furthermore, the observed elevation in antibody responses was closely associated with a sharp increase in fecal abundance of bacteria of the order Actinomycetales.

Conclusions: The results of this exploratory analysis support further investigation of molecular mechanisms involved in the contribution of PPIs to celiac disease risk through the potential enhancement of gluten immunopathology and changes in gut microbial population.

Abbreviations: HLA = human leukocyte antigen, PPI = proton pump inhibitor, TG2 = transglutaminase 2.

Keywords: antibody, celiac disease, gluten, host–microbe interaction, microbiome, proton pump inhibitor

1. Introduction

Celiac disease is an immune-mediated enteropathy characterized by inflammatory responses to the ingestion of wheat gluten and related proteins of rye and barley, which lead to lymphocytic infiltration and villous damage in the small intestine.[1] It affects approximately 1% of the population.[2] The disease is strongly associated with genes for the class II human leukocyte antigens (HLA) DQ2 and DQ8, which can present immunogenic

sequences of gluten proteins to T cells in the small intestine.[3] Transglutaminase 2 (TG2) is an important player in the disease process, both as a deamidating enzyme that enhances the immunostimulatory effect of gluten and as the target autoantigen in the ensuing immune response.[4] The predominant antibody responses in patients with celiac disease target the TG2 autoantigen, as well as native and deamidated gluten protein sequences. Among these, the IgA anti-TG2 antibody is considered to be the most sensitive and specific serologic marker of celiac disease and is used extensively to aid diagnosis.[5] Measurement of immunoglobulin G (IgG) antibody to deamidated gluten proteins also has some diagnostic utility, particularly in cases of immunoglobulin A (IgA) deficiency.[6]

Recent studies point to a rising prevalence of celiac disease in the past few decades,[7–10] although the contributing factors have not been clearly delineated. Possible proposed contributors have included increased hygiene, specific infectious agents, and changing diet or use of certain drugs that can impact the gut microbial population.[11,12] Gut microbial dysbiosis has been a significant research focus as a potential contributing factor in the onset of celiac disease in the past decade. A number of studies have reported varying degrees of microbial dysbiosis in association with celiac disease by studying the bacterial communities in saliva, intestinal tissue, and fecal samples.[13] Furthermore, antibiotic use has been implicated as a possible risk factor for the development if celiac disease, possibly due to the effect of these medications on the intestinal microbiome.[14] However, these studies are not able to link the microbial dysbiosis to a causative role in celiac disease. While the degree to which the identified dysbiosis contributes to celiac disease remains open to debate, there is evidence that gut microbial changes relevant to celiac disease can enhance gluten immunogenic potential and upregulate inflammatory pathways in the gut.[15,16]

A population-based case-control study in 2014 examined the potential link between the use of proton pump inhibitors (PPIs) and subsequent development of celiac disease.[17] The study, which included 2934 patients with celiac disease and 14,584 matched controls identified a strong association between the prescription of PPIs and the subsequent diagnosis of celiac disease (odds ratio 4.79; 95% confidence interval [CI] 4.17–5.51), with the effect being observed in both sexes and across all age strata.[17] The study did not examine mechanisms responsible for the identified link, but the authors speculated that potential alterations in the gut microbiome caused by PPIs may modulate the inflammatory response in the mucosa to promote the development of celiac disease in susceptible individuals.

Recent studies have retrospectively examined the microbiome in the context of PPI use and found significant changes in the gut bacterial population of adult PPI users in comparison with PPI non-users.[18,19] In a recent prospective study, we further examined the effect on gut microbiome through a crossover clinical trial of PPIs in a cohort of healthy individuals.[20] While the study did not detect a significant change in overall fecal microbial diversity beyond baseline variability, it showed that high-dose PPI treatment significantly affected certain taxa and was associated with an increase in genes involved in bacterial invasion of epithelial cells. Such changes may be relevant to the pathogenic mechanism of celiac disease where intestinal barrier function plays a key role in the onset of enhanced immune reactivity towards ingested gluten.[21,22]

In the current study, we aimed to examine the potential link between PPI use and enhanced immunologic reactivity to gluten and/or celiac disease autoimmunity by performing a post hoc analysis of biospecimens from our previous trial of PPI in healthy individuals.[20] The results offer novel insights that inform further approaches for examining the relationship between PPIs, gastrointestinal inflammation, and potential onset of celiac disease autoimmunity.

2. Methods

2.1. Subjects and trial design

The original study participant recruitment and trial design have been previously discussed in detail,[20] and are only described briefly here. Participants included 12 healthy volunteers 18 years or older (9 women; mean age 39.7 ± 11.1 years [standard deviation]; mean body mass index [BMI] 28.8 ± 8.5 kg/m^2) who had been screened to exclude those with known or recent gastrointestinal conditions, such as chronic gastrointestinal mucosal disease and abnormal bowel frequency, as well as those who used PPIs in the past 2 years. Study participants did not report a family history of celiac disease. Individuals in the study began taking a PPI (Omeprazole; 40 mg twice daily) at baseline (week 0) and continued for 4 weeks. After 4 weeks, half of the participants were randomized to continue with PPIs and half to discontinue until week 8. Serum and fecal samples from study participants were collected at baseline, week 4, and week 8, and stored at −80°C to maintain stability. All samples were collected with written informed consent under an institutional review board-approved protocol. This study was approved by the Institutional Review Board of Columbia University Medical Center.

2.2. Microbiome analysis

Stool sample preparation, sequencing of the 16S ribosomal RNA gene V4 region using the Illumina MiSeq 300PE platform (Illumina, San Diego, CA), and data processing and analysis have been described in detail.[20]

2.3. Analysis of celiac disease serologic and genetic markers

All serum samples were tested for antibodies to transglutaminase 2 (TG2) and to deamidated gliadin, as we have previously described.[23,24] Participants with elevated antibody to TG2 or deamidated gliadin were genotyped for celiac disease-associated HLA-DQ2 and -DQ8 alleles as described.[25,26]

2.4. Statistical analysis

Statistical analysis and interpretation of the microbiome data have been described before.[20] Analysis of the serologic data was performed with Prism 6 (GraphPad, San Diego, CA) and Minitab 17 (Minitab, Chicago, IL) software. Cohort responses to PPI administration were assessed by the Wilcoxon matched-pairs test and Welch t test. P values were 2-sided and differences were considered statistically significant at $P < .05$.

3. Results

Prior to the start of trial, serum samples from all study participants were found to be negative for IgA antibody to TG2 and IgG/IgA antibodies to deamidated gliadin according to the cutoff designated by the assay manufacturer (Fig. 1). Changes
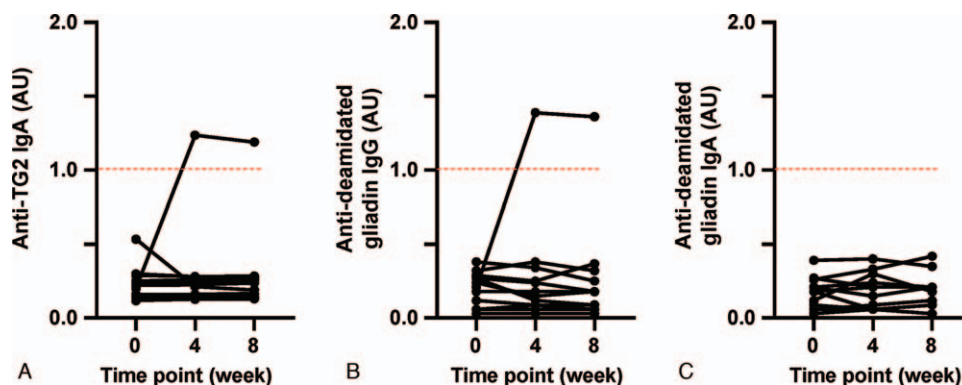
Figure 1. Celiac disease serology data in study participants (n = 12) at baseline (week 0), 4 weeks, and 8 weeks during the trial. (A) Anti-TG2 IgA antibody. (B) Anti-deamidated gliadin IgG antibody. (C) Anti-deamidated gliadin IgA antibody. Each individual is represented by a dot and the points corresponding to the same individual are connected by a line. The dotted red line represents the cutoff for positivity as set by assay manufacturer.

in levels of these antibodies for the entire cohort did not reach statistical significance at the 4- or 8-week period after the start of the trial. Furthermore, the antibody changes in the participants on 4 weeks of PPI were not significantly different from those on 8 weeks of PPI.

However, following 4 weeks of PPI, 1 individual developed a substantial increase in both anti-TG2 IgA and anti-deamidated gliadin IgG antibodies to levels above positivity (Fig. 1A and B). The IgA antibody response to deamidated gliadin did not change substantively in any individual (Fig. 1C). The individual with increased anti-TG2 IgA and anti-deamidated gliadin IgG antibodies was randomized to continue the PPI treatment for an additional 4 weeks (8 weeks total), after which the elevated antibody responses were found to have persisted (Fig. 1). Genotyping indicated that the study participant exhibiting an increase in anti-TG2 IgA and anti-deamidated gliadin IgG at the 4- and 8-week time points was positive for both HLA-DQ2 and -DQ8 genotypes. Approximately, 95% of celiac disease patients carry HLA-DQ2 and/or -DQ8, compared with an estimated 40% of the US general population.[27]

The individual with celiac disease-associated genetic susceptibility and serology was a 28-year-old woman (BMI 21.9 kg/m^2) of mixed race/ethnicity who reported loose stools and occasional abdominal cramping during the course of the study. After being

Figure 2. Relative abundance of bacteria of the Actinomycetales order in fecal sample from the individual with elevated anti-TG2 IgA and anti-deamidated gliadin IgG antibodies at baseline (week 0), 4 weeks, and 8 weeks during the trial.

informed of her elevated IgA anti-TG2 antibody following the completion of the study, she declined an offer of upper endoscopy to assess small intestinal pathology associated with celiac disease. However, she began a gluten-free diet and subsequently reported complete resolution of symptoms.

Alterations in the fecal microbiome associated with the PPI trial have been discussed in detail in Freedberg et al.[20] We further examined microbial changes for the specific individual with positive celiac disease serology after PPI use and positive HLA-DQ2 and -DQ8 genotypes. By far, the most substantive change in gut microbiome taxa was found at the level of the order Actinomycetales after 4 weeks of PPI treatment (121-fold change), which closely followed the same trend as the levels of anti-TG2 IgA and anti-deamidated gliadin IgG at 0 to 8 weeks for this individual (Fig. 2).

4. Discussion

Recent epidemiologic data have pointed to a significant association between PPI use and celiac disease diagnosis,[17] although the mechanisms contributing to this relationship are not known. In this study, we took advantage of the availability of biospecimens from a previous trial of high dose PPIs[20] to examine the possibility of enhanced gluten-associated immuno-pathology and celiac disease autoimmunity. The fact that there was no significant change in the immune response to gluten and celiac disease-associated serology for the cohort of 12 study participants was not surprising, considering the sample size and the fact that approximately 1% of the general population develops celiac disease. However, 1 of the 12 individuals in this study developed a marked increase for the celiac disease-specific autoantibody response to TG2 in conjunction with enhanced immune reactivity to deamidated gluten following PPI treatment. The individual was also found to be positive for both HLA-DQ2 and -DQ8 genotypes, at least one of which is almost always required for the development of celiac disease.[6]

The original analysis of changes in microbiome in response to the PPI trial found no significant within-individual difference in microbiome diversity, as assessed by the within-individual difference in Shannon index of diversity.[20] However, there were significant changes during PPI use in specific taxa, including Enterococcaceae, Streptococcaceae, Clostridiales, Micrococca-ceae, and Staphylococcaceae; alterations that have been previously associated with *Clostridium difficile* infection and
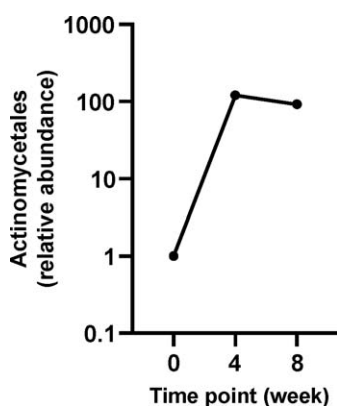
bacterial overgrowth. The observed sharp increase in Actinomycetales abundance, in conjunction with a similar elevation in serologic markers of celiac disease in the identified individual is intriguing and warrants further examination. A recent study found Actinomycetales to be associated with first degree relatives of celiac disease patients,[28] a unique cohort at risk of celiac disease that is not as confounded by potential dietary alterations as previously studied cohorts of diagnosed celiac disease patients.

It is important to note that caution should be taken in the interpretation of these results, as this exploratory study only examined serology without assessing intestinal pathology in a relatively small number of study participants. Furthermore, a weakness of the microbiome data used here is that they are derived from fecal samples. Clearly, there are significant differences between the fecal microbiome, which is a better representation of the colonic ecosystem, and the small intestinal microbiome, which is perhaps more likely to have an impact on celiac disease immunopathology. Nevertheless, in combination with findings from the earlier population-based study,[17] these observations support more in depth investigations of the mechanism(s) in the contribution of PPIs to celiac disease risk, including their potential impact on specific relevant components of the microbiome, immune reactivity to dietary gluten, and upregulation of celiac disease-associated gut inflammatory response.

Author contributions

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Analysis and interpretation of data: Sophie Jang, Julian A. Abrams, Benjamin Lebwohl, Peter H.R. Green, Daniel E. Freedberg, Armin Alaedini.

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References

[1] Alaedini A, Green PH. Narrative review: celiac disease: understanding a complex autoimmune disorder. Ann Intern Med 2005;142:289–98.

[2] Singh P, Arora A, Strand TA, et al. Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol 2018;16:823.e2–36.e2.

[3] Louka AS, Sollid LM. HLA in coeliac disease: unravelling the complex genetics of a complex disorder. Tissue Antigens 2003;61:105–17.

[4] Yu XB, Uhde M, Green PH, et al. Autoantibodies in the extraintestinal manifestations of celiac disease. Nutrients 2018;10:1123.

[5] Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1981–2002.

[6] Briani C, Samaroo D, Alaedini A. Celiac disease: from gluten to autoimmunity. Autoimmun Rev 2008;7:644–50.

[7] Rubio-Tapia A, Kyle RA, Kaplan EL, et al. Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology 2009;137:88–93.

[8] Catassi C, Kryszak D, Bhatti B, et al. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. Ann Med 2010;42:530–8.

[9] Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther 2007;26:1217–25.

[10] Riddle MS, Murray JA, Porter CK. The incidence and risk of celiac disease in a healthy US adult population. Am J Gastroenterol 2012;107:1248–55.

[11] Welander A, Tjernberg AR, Montgomery SM, et al. Infectious disease and risk of later celiac disease in childhood. Pediatrics 2010;125:e530–6.

[12] Zhernakova A, Kurilshikov A, Bonder MJ, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 2016;352:565–9.

[13] Cenit MC, Olivares M, Codoner-Franch P, et al. Intestinal microbiota and celiac disease: cause, consequence or co-evolution? Nutrients 2015;7:6900–23.

[14] Marild K, Ye W, Lebwohl B, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case-control study. BMC Gastroenterol 2013;13:109.

[15] Galipeau HJ, McCarville JL, Huebener S, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am J Pathol 2015;185:2969–82.

[16] Caminero A, McCarville JL, Galipeau HJ, et al. Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2. Nat Commun 2019;10:1198.

[17] Lebwohl B, Spechler SJ, Wang TC, et al. Use of proton pump inhibitors and subsequent risk of celiac disease. Dig Liver Dis 2014;46:36–40.

[18] Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740–8.

[19] Takagi T, Naito Y, Inoue R, et al. The influence of long-term use of proton pump inhibitors on the gut microbiota: an age-sex-matched case-control study. J Clin Biochem Nutr 2018;62:100–5.

[20] Freedberg DE, Toussaint NC, Chen SP, et al. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. Gastroenterology 2015;149:883–5.

[21] Schumann M, Siegmund B, Schulzke JD, et al. Celiac disease: role of the epithelial barrier. Cell Mol Gastroenterol Hepatol 2017;3:150–62.

[22] Volta U, De Giorgio R, Caio G, et al. Nonceliac wheat sensitivity: an immune-mediated condition with systemic manifestations. Gastroenterol Clin North Am 2019;48:165–82.

[23] Huebener S, Tanaka CK, Uhde M, et al. Specific nongluten proteins of wheat are novel target antigens in celiac disease humoral response. J Proteome Res 2015;14:503–11.

[24] Uhde M, Ajamian M, Caio G, et al. Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. Gut 2016;65:1930–7.

[25] Lau N, Green PH, Taylor AK, et al. Markers of celiac disease and gluten sensitivity in children with autism. PLoS One 2013;8:e66155.

[26] Moeller S, Lau NM, Green PH, et al. Lack of association between autism and anti-GM1 ganglioside antibody. Neurology 2013;81:1640–1.

[27] Kagnoff MF. Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest 2007;117:41–9.

[28] Bodkhe R, Shetty SA, Dhotre DP, et al. Comparison of small gut and whole gut microbiota of first-degree relatives with adult celiac disease patients and controls. Front Microbiol 2019;10:164.