Celiac disease serology and gut microbiome following protein pump inhibitor treatment

Sophie Jang, MS\(^{a,b}\), Benjamin Lebwohl, MD\(^{a,c,d}\), Julian A. Abrams, MD\(^{a,d}\), Peter H.R. Green, MD\(^{a,c}\), Daniel E. Freedberg, MD\(^{a,e}\), Armin Alaedini, PhD\(^{b,c,e,f}\)

**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 protein 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 microbiotal 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, protein 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

---

Editor: M. Scott Killian.

This study was supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health (NIH), through Grant Number UL1 TR000040 (AA). DEF was funded in part by NIH K23 DK111847 and a Research Scholar Award from the American Gastroenterological Association. The funding agencies had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

The authors declare no competing interests.

Data Sharing Plan: All of the experimental methods and generated data will be shared in a transparent fashion with investigators requesting such information.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

\(^{a}\) Department of Medicine, \(^{b}\) Institute of Human Nutrition, \(^{c}\) Celiac Disease Center, \(^{d}\) Department of Epidemiology, Columbia University Irving Medical Center, \(^{e}\) Department of Medicine, New York Medical College, Valhalla, New York.

Correspondence: Armin Alaedini, Department of Medicine, Columbia University Medical Center, 1130 Saint Nicholas Ave., New York, NY 10032 (e-mail: aa819@columbia.edu); Daniel E. Freedberg, Department of Medicine, Columbia University Medical Center, 622 W. 168th Street, New York, NY 10032 (e-mail: def2004@cumc.columbia.edu).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Jang S, Lebwohl B, Abrams JA, Green PH, Freedberg DE, Alaedini A. Celiac disease serology and gut microbiome following protein pump inhibitor treatment. Medicine 2020;99:35(e21488).

Received: 22 August 2019 / Received in final form: 18 April 2020 / Accepted: 28 June 2020

http://dx.doi.org/10.1097/MD.0000000000021488
sequences of gluten proteins to T cells in the small intestine.\(^{[1]}\) 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 ± 8.3 kg/m²) 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
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.

The individual with celiac disease-associated genetic susceptibility and serology was a 28-year-old woman (BMI 21.9 kg/m²) of mixed race/ethnicity who reported loose stools and occasional abdominal cramping during the course of the study. After being 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. 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, 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 to examine the possibility of enhanced gluten-associated immunopathology 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.

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. However, there were significant changes during PPI use in specific taxa, including Enterococcaceae, Streptococcaceae, Clostridiales, Micrococcaeae, 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, 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, these observations support more in depth investigations of the mechanism(s) in the contribution of PPIs to celiac disease risk, including their potential in the earlier population-based study, 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**


**References**
