COVER PAGE

Official Title of the Study: Tissue destruction and healing in celiac disease
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1. Background

Celiac disease (CeD) is an autoimmune-like small intestinal enteropathy induced by dietary gluten in genetically susceptible HLA-DQ2 or DQ8 individuals. There is a spectrum in tissue damage: potential CeD patients develop inflammatory IFN-γ anti-gluten T cell immunity in the absence of tissue damage, while active CeD is associated with tissue damage ranging from partial to total villous atrophy (VA). Gluten-free diet (GFD) is to this date the only treatment for CeD. However, 50% of CeD patients are unable to effectively adhere to a diet that sustainably excludes gluten, suffering therefore from inadvertent gluten exposure. Moreover, more than 30% of CeD patients have persistent high symptom burden, resulting from continued mucosal damage despite adhering to a GFD.

Persistent mucosal damage on a GFD is associated with several severe complications, including lymphoproliferative malignancy and bone diseases. In addition, patients with active CeD display a wide range of clinical presentations, including metabolic defects (vitamin, iron, cholesterol) that are not correlated to the degree of VA. Although much progress has been made in understanding CeD, major gaps remain in biologic mechanisms underlying interindividual differences in clinical presentations and capacity to heal on a GFD. In CeD, there is no data available on gene expression variation in the targeted tissue across the disease spectrum. There are publications on a subset of key individual immune cell types, but we lack a global analysis of all immune cell subsets present in the intestinal mucosa. Furthermore, while there is evidence for a role of the microbiome in CeD, we lack information on the duodenal mucosal-associated and luminal microbiota, which are most likely to directly interact with the immune system and have metabolic effects. Finally, very little is known about interactions between intestinal epithelial cells (IECs), immune cells and the microbiota in CeD, and how they are linked to gluten and the different CeD clinical phenotypes.

To address these gaps, we have assembled a team of internationally recognized experts in the field of CeD, mucosal immunology and microbiome. The RC2 proposal is anchored around multi-omics studies performed in the context of longitudinal interventional gluten challenge and de-challenge studies, on ***180*** clinically well-characterized CeD patients and **40** HLA-matched healthy controls.

2. Purpose

The purpose of this study is to: (i) address critical gaps in our understanding of CeD pathogenesis and clinical presentations, (ii) test the hypothesis that interactions between IECs, microbiota, immune system, genetics and gluten underlie differences in clinical presentation, severity of tissue destruction, and ability to heal (iii) generate resources and hypotheses to advance patient care.

Our aims are:

Aim 1: ***Gluten challenge studies to gain a mechanistic understanding of tissue destruction***. We will perform longitudinal gluten challenge studies combined with integrated multi-omics investigation on a well-curated cohort of 90 GFD patients. Each patient will undergo three endoscopies. Studies will combine epithelial, microbial, genetic, metabolic and immunological analyses, and yield a large diverse set of biobanked samples. We will characterize the molecular, epithelial metabolomic and metagenomics signature of tissue destruction and develop a predictive model of tissue destruction. Extensive clinical metadata and histological data will be obtained at all three time points.

Aim 2: ***Gluten de-challenge studies to gain a mechanistic understanding of tissue healing*****.** Through longitudinal gluten de-challenge studies with three endoscopies that will include 90 patients, we will characterize the molecular, epithelial, genetic, metabolomic and metagenomics signature of tissue healing and develop a predictive model of tissue healing. This study will also yield a large diverse set of biobanked samples. Extensive clinical metadata and histological data will be obtained at all three time points.

Aim 3: Developing research resources for the scientific and medical community to advance patient care and hypothesis-generating science. We will collect a large array of biosamples (580 biopsy DNA and RNA samples, 580 PBMCs and serum samples, 580 frozen biopsies for bacterial cultures, 580 frozen biopsies for host-bacterial imaging, 580 urine and saliva samples, 400 intestinal organoids). Together, the U Chicago and Mayo Clinic biobanks will have ***~4640*** biobanked samples. In addition, a large resource of multi-omics data sets will be made available to the research community (~**2790**) in Aims 1 and 2. To integrate such large and complex datasets, we will build an integrated clinical research data management and informatics platform that will support harmonization, curation, dissemination, and analysis of data collected at various levels. All these data will be made publicly available and searchable using a user-friendly web platform. Together, this integrated clinical research data management, biobank and informatics platform will constitute a powerful resource for the scientific and medical community.

This study, in addition to generating large integrated omics data sets and hypotheses on mechanisms underlying tissue destruction and healing, will develop tools to improve the clinical follow-up of CeD patients, and resources for the scientific and medical community to advance CeD research and clinical care.

3. Methodology

a. Study overview. To understand the mechanisms underlying tissue destruction and healing we have designed longitudinal challenge and de-challenge studies with 90 patients in each interventional arm. In addition, 40 MHC-matched healthy controls will be enrolled and undergo one endoscopy with samples collection. Clinical data and omics data will be obtained at each endoscopy, and samples will be collected for biobanking (**see Fig. 1**). Subjects will be recruited

equally at the University of Chicago and Mayo Clinic, Rochester. The fully Schedule of Events can be found in Appendix A.

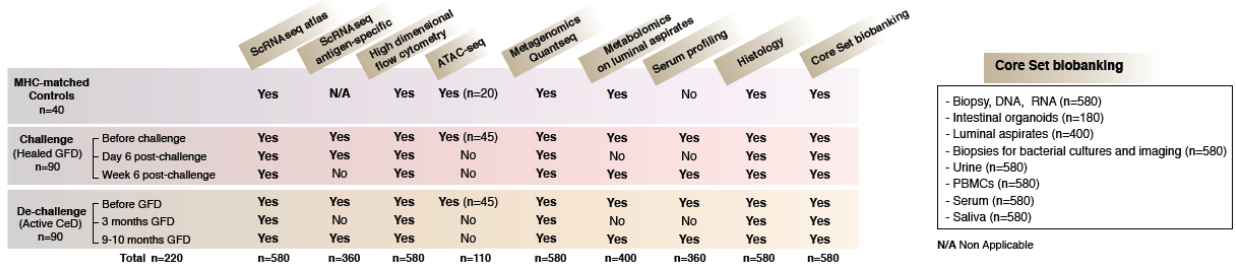

Fig. 1: RC2 data collection and biobanking

b. Longitudinal challenge study.

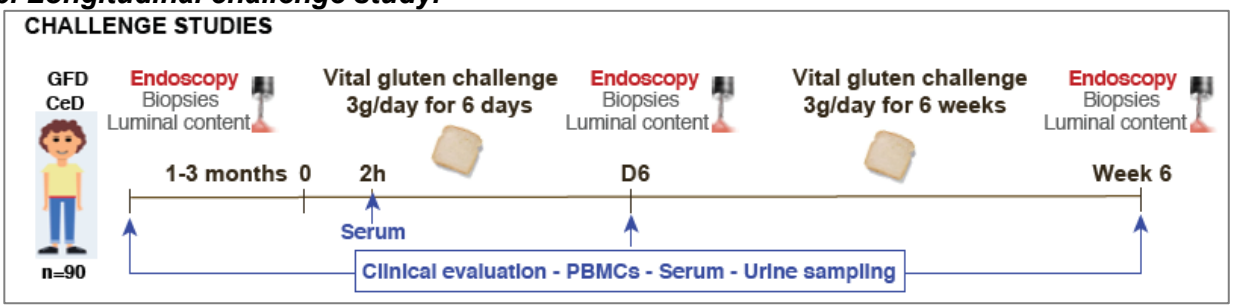

Fig. 2: Vital gluten challenge studies. 90 GFD patients will be challenged for a total of 6-weeks. 3 endoscopies at the indicated time points will be performed. For analyses and biobanking see **Fig.1**

In the gluten challenge study arm (**Figure 2**), 90 celiac disease patients (45 from University of Chicago; 45 from Mayo Clinic) will be enrolled. After informed consent, patients will have blood drawn for screening labs. Qualifying patients will be asked to return to undergo a research endoscopy with biopsies and luminal content sampling. After confirmation that their histology is normal, the subjects will proceed to a gluten challenge with 3g vital gluten per day. On day 6 after starting the challenge, a research endoscopy with be performed with biopsies and luminal content sampling. Subjects will continue on 3g vital gluten each day for 6 weeks. At the end of study, patients will undergo a research endoscopy with biopsies and luminal content sampling. In addition to research endoscopies, patients will have saliva, serum and urine sampling at baseline, 2 hour after first gluten ingestion on day 6 and end of study.

Gluten challenge will be provided to subjects in the form of a 3g vital gluten bar. Subjects will be instructed to ingest the gluten with the same meal every day. Phone calls will be performed weekly during the gluten challenge period to: 1) conduct symptom review using the ICDSQ and assign severity score, 2) assess compliance with gluten (must be 80% or higher), 3) assess adverse events and 4) remind patient of next week's call.

c. Longitudinal de-challenge study

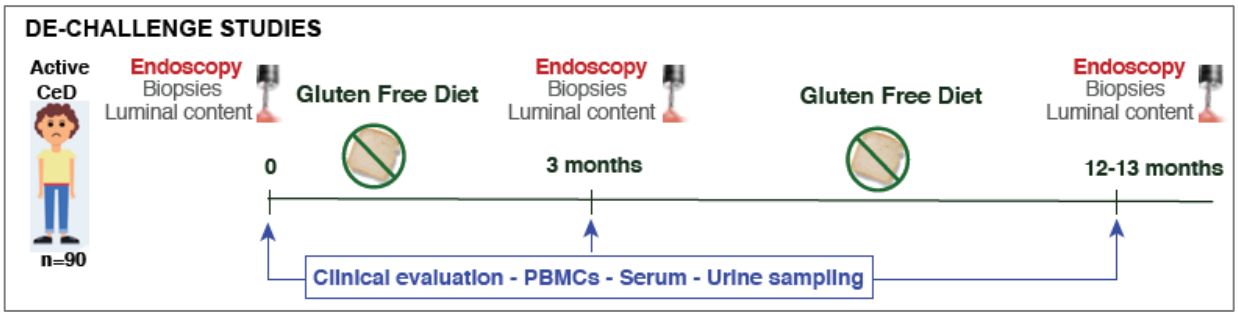

Fig. 3: Gluten de-challenge studies. De-challenge studies will be performed in 90 active CeD patients. 3 endoscopies at the indicated time points will be performed. For analyses and biobanking see **Fig.1**

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In the gluten de-challenge study arm (**Figure 3**), 90 newly diagnosed celiac disease patients (45 from University of Chicago; 45 from Mayo Clinic) will be enrolled. Interested patients will be asked to sign the informed consent. During a routine endoscopic procedure, 14 additional biopsies will be taken and luminal content will be sampled for study purposes. Subjects will receive education about the gluten free diet from a registered dietician at University of Chicago and Mayo Clinic as part of routine care. A research endoscopy will be performed at 3 months after starting the gluten free diet and 12-13 months after starting the gluten free diet. In addition to research endoscopies, patients will have serum and urine sampling at baseline, 3 months and 12-13 months after starting the gluten free diet. Patients will receive monthly phone calls during the study period to check on celiac symptoms and diet adherence. Additionally, subjects will be reminded to fill out the study questionnaires measuring symptoms, quality of life and diet adherence.

d. Control. Forty healthy controls (20 from University of Chicago; 20 from Mayo Clinic Rochester) will be recruited from the local population by advertisement. After informed consent, control subjects will have blood drawn for screening labs. Qualifying subjects will be asked to return to undergo a research endoscopy with biopsies and luminal content sampling, as well as saliva, serum and urine sampling. A phone call 1 day after the endoscopy will be performed to assess for any adverse events related to the study endoscopy. The CDSD will also be administered to ensure to baseline symptoms in this control population.

Controls will be broadly matched to the cases by sex and age (+/- 20 years). Controls must be matched by HLA class II tissue typing. Tissue typing will be done at the first visit for controls only. If HLA typing does not match celiac patients, the matched control will not continue in the study past the first visit. The selection criteria are open to all races and both genders, so long as females are not pregnant. Only subjects with conditions that may have an impact on the aims of the study are excluded. No other exclusions will be applied. Due to the demographics of celiac disease, we do not expect the minority population to exceed 2% black, 2% Asian, 4.5% Hispanic, and 4.5% 'other' for an ideal target of 13% minority participation. Due to non-Hispanic Whites having the highest prevalence of celiac disease, discrepancies may occur.

e. Gluten Challenge Bar

The Gluten challenge food article will be made in a commercial kitchen (Mayo Clinic Food Services, Gluten Free Kitchen; address: Professional Building Campus, 41st Street NW Rochester MN 55904). Ingredients will be included like common food ingredients. Vital wheat gluten will be incorporated into a mix that is otherwise free of gluten.

This will be a commercial grade food article designed to be palatable (especially important for study adherence given daily ingestion for 6 weeks) and to contain 3 grams of gluten. This gluten containing food will have the following characteristics:

- Low in FODMAPs (Fermentable oligosaccharides, disaccharides, monosaccharides and polyols) to reduce any IBS like symptoms that FODMAPs might induce symptoms in susceptible individuals.
- Prepared in Gluten free kitchen from GF ingredients except for the addition of vital wheat Gluten.
- Individually wrapped with nutritional information label
- Controlled for common allergens
- Able to be reduced in size with predictable gluten content in case dose reduction is needed.
- Designed to be frozen (up to 3 months with optimal taste and 12 months to food safety) for long term storage
- To be thawed before consuming and consume within 4 days of thawing

Bars will be shipped frozen to the University of Chicago. Bars will be provided frozen to study participants.

f. *Symptom Diary and Biospecimens*

Symptom & Gluten free adherence Surveys
Symptoms will be assessed using the validated CDSD and ICDSQ. The daily CDSD is a 5-item survey that assesses for symptoms of diarrhea, abdominal pain, bloating, nausea and fatigue using a 5-point scale. The CDSD will be administered once at time of screening, once at time of endoscopy and then daily beginning day 0 through the end of the study period. If subjects rate symptoms as “severe” or “very severe”, the research coordinator will discuss with the PI regarding continuation in the study.

The ICDSQ is a 14-item survey that is administered on a weekly basis and assesses the impact of CeD symptoms on subjects’ quality of life. If the subject reports significant impact of symptoms on quality of life and daily functioning, the research coordinator will alert the PI to discuss continuation in the study. Subjects will be asked to fill out the ICDSQ at the end of the day.

The CDAT is a 7-question survey assessing gluten free diet adherence. The CDAT will be administered to both the challenge cohort (to evaluate gluten free diet outside of the challenge bar) and de-challenge cohort at time of screening, first endoscopy and then at each endoscopy visit (day 6 and week 6).

The IGFDQ survey is a 20-item survey covering adherence questions and will be administered to the de-challenge cohort to assess adherence to the gluten free diet at each study visit (screening, 3 months, and 12 months).

All of the surveys will be administered electronically through RedCap.

Saliva, Blood and Urine samples

The challenge and control groups will have blood collected for initial screening labs prior to their first endoscopic procedure. The dechallenge group does not undergo this initial screening lab because this is typically already completed as part of routine care.

Saliva blood and urine samples will be obtained at every study visit after that. For the challenge cohort, biospecimens are collected prior to gluten challenge (screening period), at 2 hours post challenge, 6 days post challenge, and 6 weeks post challenge. For the de-challenge cohort, biospecimens are collected at baseline, 3 months after starting gluten free diet and 12-13 months after starting gluten free diet. For the control group, biospecimens are collected once at the time of endoscope.

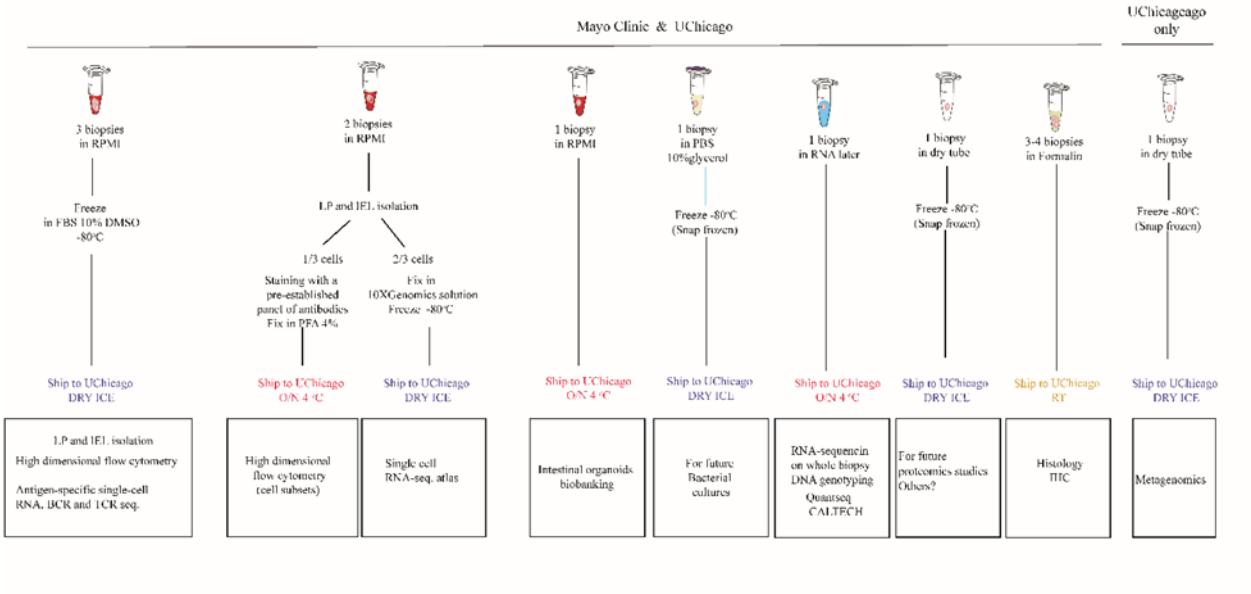
Upper Endoscopy
Three upper endoscopies will be performed for participants in the gluten challenge and de-challenge cohorts. One endoscopy will be performed in the healthy control cohorts. For the gluten challenge cohort, endoscopy is performed at screening, 6 days of gluten challenge, and 6 weeks of gluten challenge. For the de-challenge cohort, endoscopy is performed at screening (routine care), 3 months and 12-13 months after starting the GFD. For the healthy control cohort, endoscopy is performed once at baseline.

The upper endoscopies will be carried out by the standard protocol in place at each of the medical centers. Typically, patients will be in the left lateral decubitus position. Two liters of oxygen will be given by nasal cannula. Vital signs oximetry and respiration will be monitored continuously throughout the procedure. Adults will be given moderate sedation most typically with intravenous fentanyl, midazolam with the occasional addition of topical anesthesia or diphrenhydramine as per endoscopist's discretion and history of prior sedation if any. In select patients who have a history of difficult sedation or are otherwise deemed to require higher level of sedation, monitored anesthesia care (MAC) will be used. Participants will be monitored after the procedure for at least 60 minutes and until the following criteria are met: alert and oriented, able to protect airway, stable vital signs over the 30 minutes, normal cardiac rhythm, heart rate and pulse within 20 points of baseline or within normal limits, oxygen saturation 95% or greater, afebrile, ambulation at baseline. The subject must have a responsible adult to provide transportation upon dismissal from the unit. Precautions in place for Covid-19 pandemic protection will be followed in line with contemporary guidance in place at the time of endoscopy.

Small bowel biopsies
Research biopsies: Up to14 biopsies will be collected for research purposes. Luminal aspirates from the second portion of the duodenum will be obtained during the upper endoscopy. These biopsies will be placed in the requisite media and shipped to the University of Chicago.

Of the research biopsies that will be taken from patients, three to four may be used for histopathology, three will be used for analysis of antigen-specific T and B cells studies, one for single cell RNA seq, one for high dimensional flow cytometry, one in RNA-later for whole biopsy RNA sequencing (RNA-seq), one for preparation of intestinal organoids, and three frozen (one for proteomic studies, one for metagenomics, and one for bacterial cultures). The detailed information is in the figure below (Figure 4). Of 10 research biopsy samples, 6 biopsy samples will be put in the RPMI media, one biopsy in RNA later, three biopsy samples will be frozen instantly (snap freezing), and three-four biopsy samples will be placed in formalin.

Figure 4. Small Intestinal Biopsies



g. Metadata will be collected on all study subjects at baseline and after intervention. To clinically phenotype CeD patients, we will employ a standardized and validated patient-reported symptom tool called the Celiac Disease Symptom Diary (CDSD) that assesses for presence, frequency and severity of classical and non-classical symptoms of CeD. The CDSD is the current gold standard tool in therapeutic drug trials in CeD based on the fact that it follows FDA guidance on patient-reported outcome. CeD patients will be stratified as classical, non-classical or asymptomatic according to their CDSD responses. We anticipate that the breakdown of clinical phenotypes will be: ~25% classical, ~55% non-classical and ~20% asymptomatic. To assess the clinical metabolic profile, we will analyze lipid, vitamin and metal profiles. The harmonized clinical data will be entered into a shared REDCap database linked with experimental data.

This study will use the following information from subject self-report and medical record extraction (note data sharing outside the study team is detailed separately below):

- Name
- MRN
- Race and ethnicity
- Date of Birth
- Medical history
- Medication history
- Lab results (both clinical labs and research lab analysis)
- Pathology reports
- Imaging result
- Endoscopic results
- ICDSQ Data
- CDAT Data
- IGFDQ Data
- Dates of significant clinical events (dates of enrollment, consent, endoscopy procedures, diagnosis, lab results, etc)

h. Histology analysis: Any histology analysis required for routine care will be conducted at the respective site according to routine clinical practices. Histology performed for research purposes will be processed at the University of Chicago. Histology images will be uploaded in the Integrated Web-based clinical research and data management platform and villus height to crypt depth ratio (Vh:Cd) will be quantified centrally in a blinded manner by Dr**.** Weber at the University of Chicago. Unstained sections will be shared with the team for validation approaches.

i. Sample collection, analysis, and biobanking**:** Whole biopsy RNA sequencing (RNA-seq), single cell RNA-seq (scRNA-seq), high dimensional flow cytometry and metagneomics will be performed on all patients and all time points. Single cell ATACseq (scATAC-seq) for epigenetic profiling will be performed on 40 controls, 45 challenge and 45 dechallenge patients at the time of the first endoscopy. Analysis of antigen-specific T and B cells will be performed at the 1st and 2nd endoscopy for the challenge study and the 1st and last endoscopy of the de-challenge study (**Fig. 1**). The workflow is organized in such a way that all experiments will be performed at U Chicago. Mayo clinic will be in charge of isolating cells from biopsies. Isolated cells will be fixed for scRNA seq, and stained with an antibody panel provided by U Chicago and fixed before shipment. Mayo Clinic will also put one biopsy in RNA later for transcriptional profiling and send one biopsy to U Chicago for the generation of intestinal organoids that will be stored for future studies. In addition, both U Chicago and Mayo Clinic will prepare one biopsy for metagenomics analysis and ship DNA to Caltech for analysis. Core set biobanking will be performed on all patients (**Fig. 1**). The banking of all samples is described below under each participating institution.

Clinical coordinators, technicians and investigators from Mayo Clinic and U Chicago will be trained by U. Chicago investigators, and will follow SOPs for sample collection and shipment. Mayo Clinic will receive kits with instructions, barcodes, and labeled tubes for sampling and shipment.

General sample management will be as follows:

University of Chicago:
The UofC team will bank blood, urine and tissue from both UofC and Mayo. Any samples shared from Mayo will be labelled with the subject's study number and will not contain any other identifiers. UofC will conduct genetic analysis for study purposes and will store DNA and RNA. These samples will be stored indefinitely for future research purposes. These samples can be shared in accordance with the sharing process detailed below.

Mayo Clinic
The team at Mayo will bank the PBMCs and serum samples for this study. The UofC team will process blood samples from UofC subjects and send PBMCs and serum samples to Mayo. These samples will be labelled with the subject study code only and will not contain any identifiers. Additionally, Mayo will process and store PBMC and serum samples from their own site. These samples will be stored indefinitely for future research purposes. These samples can be shared in accordance with the sharing process detailed below.

CalTec
The team at CalTec will receive saliva samples and tissue RNA/DNA from UofC and Mayo to perform microbiome genetic analysis and for banking purposes. The samples will be labelled

with the subject study code only and will not contain any identifiers. Left over tissue and saliva samples will be stored indefinitely for future research purposes. These samples can be shared in accordance with the sharing process detailed below.

4. Duration
The study will last for 5 years.

5. Locations and IRB Reliance

All participating centers will reply on the University of Chicago IRB. Participating centers are described below:

Institution	PI Name	Activities being conducted	IRB of Record
University of Chicago *Hyde Park and River East locations*	Sonia Kupfer. MD	Study sponsor and lead site Subject enrollment and all study activities Sample analysis and banking Retention of study data	University of Chicago
Mayo Clinic	Joseph Murray, MD	Subject enrollment and all study activities Sample analysis and banking	University of Chicago
California Institute of Technology (CalTec)	Rustem Ismagilov, PhD	Sample analysis and banking only (no subject enrollment)	University of Chicago

6. Experimental Subjects

a. number of subjects
To understand the mechanisms underlying tissue destruction and healing we have designed longitudinal challenge and de-challenge studies with 90 patients in the challenge arm (45 from University of Chicago; 45 from Mayo Clinic Rochester) and 90 patients in the de-challenge arm (45 from University of Chicago; 45 from Mayo Clinic Rochester). In addition, 40 MHC-matched healthy controls (20 from University of Chicago; 20 from Mayo Clinic Rochester) will be enrolled.

b. method of subject selection and recruitment
The clinical research associate (CRA) will identify eligible subjects via IRB-approved HIPAA-compliant methods. The CRA will review clinic and endoscopy schedules as well as the electronic medical record system for potentially eligible subjects.

The CRA will contact potential subjects in clinic or by telephone to explain the study, to review eligibility criteria and obtain informed consent. CRAs will talk with interested potential subjects to provide complete disclosure of the study objectives and procedures, potential risks and benefits, and expectations and then document the process of written consent obtained from subjects. The CRAs will be responsible for all aspects of protocol logistics for recruited subjects. This includes obtaining required demographic and questionnaire data, handling of all collected biospecimens, and ensuring smooth completion of the study requirements.

Each potential subject screened for eligibility will be identified only by sequential number, contact methods, and reason for recruitment failure or success. Recruitment numbers will be reported monthly to the site PIs to monitor accrual.

There is no randomization in this study.

c. Inclusion and exclusion criteria:

c.1. Challenge study:

Inclusion Criteria:

Subjects must fulfill all of the following criteria at screening to be eligible for participation:

1. Males or females 18 to 70 years of age, inclusive.
2. Subjects demonstrate willingness to participate in the study and to perform all required procedures as documented by signed informed consent.
3. Subjects must have a diagnosis of CeD by intestinal biopsy at least 12 months and positive celiac serology at some point in their evaluation, prior to screening as confirmed by medical records or written physician statement.
4. Subjects must have reported following a strict GFD for at least the 12 consecutive months and must be willing to maintain their current diet for the duration of study participation while being in gluten challenge.
5. Patients who don't show mucosal healing with a VH:CD ratio <2 at the baseline endoscopy will enter the GFD non-healed cohort (see below) and will not undergo gluten challenge. Ninety percent of patients who are asymptomatic, adhere to GFD and are seronegative have achieved histologic healing,

Exclusion Criteria:

Subjects are excluded from the study if any of the following criteria applies:

1. Current diagnosis of any severe complication of CeD, such as refractory CeD (RCD) type I or II, enteropathy-associated T-cell lymphoma (EATL), ulcerative jejunitis, or GI perforation.
2. Diagnosis of any chronic, active GI disease other than CeD, such as active, untreated peptic ulcer, eosinophilic esophagitis, erosive esophagitis, ulcerative colitis or Crohn's disease, microscopic colitis, irritable bowel syndrome, small intestinal bacterial overgrowth, tropical sprue, or other GI and non-GI disorder or prior GI surgery that may, in the Investigator's opinion, interfere with the assessment of symptoms of abdominal pain, diarrhea, or other components of CeD.
3. Selective IgA deficiency, defined as having undetectable levels of serum IgA.
4. History of severe reaction to gluten exposure that is considered incapacitating or life-threatening.
5. Known or suspected exposure to COVID-19 infection in the 4 weeks before the screening.
6. History or presence of any clinically significant disease that, in the opinion of the Investigator may confound the subject's participation and follow-up in the clinical trial or put the subject at unnecessary risk, including but not limited to: uncontrolled hypertension (BP ≥180/110 mm/Hg), unstable angina, Class II congestive heart failure or major fluid overload, coronary angioplasty or myocardial infarction within the past 6 months, clinically significant arrhythmias or electrocardiogram (ECG) abnormalities, severe chronic pulmonary disease, or any renal, hematologic, GI, immunologic, dermatologic, neurologic, or psychiatric disease.
7. History of significant substance or alcohol abuse during the 12 months prior to screening as obtained by medical record and/or subject report.
8. Females who are pregnant or planning to become pregnant during the study period, or who are currently breastfeeding.

9. Participation in another investigational drug or device study or treatment with an investigational drug within 30 days or 5 half-lives, whichever is longer, prior to screening.
10. Individuals with uncontrolled clotting disorders or on anti-coagulants that they can't safely hold prior to endoscopy.
11. Participation in clinical trial within 30 days prior to consenting, and 12 months for a clinical trial including a biologic agent.
12. Participation in clinical trial with tolerogenic agents

c.2. De-challenge study:

Inclusion criteria:

Subjects must fulfill all of the following criteria at screening to be eligible for participation:

1. Males or females, 18 to 70 years of age, inclusive.
2. Subjects demonstrate willingness to participate in the study and to perform all required procedures as documented by signed informed consent.
3. Newly suspected subjects of CeD, either typical symptoms of CeD or referred to celiac clinic due to positive CeD serology undergoing upper endoscopy. Patients with weak positive serology will not be included in order to maximize the likelihood of the patients qualifying for the dechallenge study.
4. Subjects who are currently not on GFD.

Exclusion Criteria:

1. Has a history of chronic inflammatory gastrointestinal disease (example, inflammatory bowel disease, extensive colitis, ulcerative jejunitis, drug induced enteropathy) (Microscopic colitis is not an exclusion criteria).
2. Has chronic infectious gastrointestinal illness, or acute infectious gastrointestinal illness within the 4-week period prior to screening.
3. Known history of lymphoproliferative disease, including monoclonal gammopathy of unknown significance, lymphoma, or signs and symptoms suggestive of possible lymphoproliferative disease, such as lymphadenopathy and/or splenomegaly.
4. Individuals with uncontrolled clotting disorders or on anti-coagulants that they can't safely hold prior to endoscopy.
5. Known or suspected exposure to COVID-19 infection in the 4 weeks before the screening.
6. History or presence of any clinically significant disease that, in the opinion of the Investigator may confound the subject's participation and follow-up in the clinical trial or put the subject at unnecessary risk, including but not limited to: uncontrolled hypertension, unstable angina, Class II congestive heart failure or major fluid overload, coronary angioplasty or myocardial infarction within the past 6 months, clinically significant arrhythmias or ECG abnormalities, severe chronic pulmonary disease, or any renal, hematologic, GI, immunologic, dermatologic, neurologic, or psychiatric disease.
7. History of significant substance or alcohol abuse during the 12 months prior to screening as obtained by medical record and/or subject report.
9. Participation in another investigational drug or device study or treatment with an investigational drug within 30 days or 5 half-lives, whichever is longer, prior to screening.
11. Participation in clinical trial within 30 days prior to consenting, and 12 months for a clinical trial including a biologic agent.
12. Participation in clinical trial with tolerogenic agents
13. Participants whose initial biopsies do not reveal villous atrophy will not be followed in the de-challenge group but may be recruited into the potential celiac disease cross sectional group so long as they meet the criteria for inclusion into that group.

c.3. Controls:

Inclusion criteria:

- Subjects (both cases and controls) will include males and non-pregnant females, aged 18-70 years.
- Females of reproductive age or capacity (i.e. not having had tubal ligation or sterilization) will have a pregnancy test performed.

Exclusion criteria:

- Subjects on antibiotics, proton pump inhibitors, aspirin, non-steroidal anti-inflammatory drugs, alcohol intake within 48 hours, a bowel preparation within 4 weeks of the studies, and smokers
- Subjects will be asked not to take any probiotics in the week before testing.
- Any known intestinal inflammation such as GERD, eosinophilic esophagitis, and inflammatory bowel disease.
- Prior gastrointestinal surgery (other than appendectomy)
- Ongoing use of antiplatelet agents or anticoagulants.
- Controls should not have a prior history of or family history of CD.
- Subjects unable to provide informed consent
- The presence of any medical or psychological condition could interfere with the safe performance of the upper endoscopy.

7. Statistical analysis

Challenge studies

a. Developing predictive models of tissue destruction. We will perform ***challenge studies*** (**Fig. 2**), which will provide unique insights into the dynamic of the host response and microbial components associated with tissue destruction. Under this experimental setting, patients will serve as their own control as they will undergo 3 endoscopies over the course of the interventional study. The flow-cytometry and scRNAseq analysis preceding gluten challenge will determine the frequency of gluten-specific T cells and the state of IECs at baseline. The 6-day analysis will give insights into the early antigen-specific T and B cells responses (quantitative and qualitative by combining scRNA-seq on fresh biopsies, flow cytometry and scRNA-seq on antigen-specific T and B cell responses). Secondly, we will investigate how early response impacts IECs, e.g. by promoting expression of non-classical MHC class I molecules MICA and HLA-E that activate NKG2D and CD94/NKG2C NK receptors expressed on cytotoxic IELs (IE-CTLs), respectively, and consequently affect the cytolytic potential and activation status of IE-CTLs that in turn mediate IECs destruction. We will link this initial response to the development of VA (we expect ~60% of patients to develop VA). This will determine whether the frequency and/or intensity of the anti-gluten T cell response drives tissue destruction. We will also determine effects that occur early in IECs after gluten challenge (6 days) and if these lead to persistent changes in stem cells that then manifest in specified conditions or lineages. We will compare single cell transcriptomes with the single cell atlas from biopsies from patients that respond to gluten with damage (60-70%) versus those that do not. Thus, we can determine the role of IECs in driving disease (not just as a passive target) prior to their damage. Furthermore, we can identify which alterations in other immune subsets (such as γδ T cells and macrophages) promote or prevent changes in IECs, IELs activation and tissue destruction. Finally, we will assess whether microbial composition and function (metagenomic and metabolomic analysis) preceding gluten challenge influences activation of gluten specific T cells, and if early microbial changes are linked to IE-CTLs activation and ultimately VA. To determine the potential impact of the microbiota on tissue destruction and healing (see de-

challenge study below), we will analyze mucosal-associated and luminal bacteria as described in ***section e***. The role of the microbiota in tissue destruction and healing is complex and needs to be integrated with the host response**.** These microbes could impact tissue destruction (and healing, see below) via three mechanisms: (i) Microbes could compete with or interfere with host nutrient uptake (e.g. by deconjugating and modifying bile acids), driving various metabolic deficiencies and therefore further weakening host antimicrobial defenses and contributing to tissue damage or interfering with tissue healing. In addition, they could produce metabolites (e.g. butyrate or modified bile acids) that promote or prevent tissue destruction by modulating immune responses; (ii) Pro-inflammatory or immunomodulatory molecules (e.g. LPS or TLR2/1 ligands or polysaccharide A, respectively), especially from mucosa-associated bacteria, can drive inflammatory or tolerogenic immune responses, that in turn promote or hamper tissue destruction. Persistence of adherent microbes after gluten removal could impact IECs function and continue to drive inflammation and prevent healing or lead to ongoing gastrointestinal symptoms. (iii) Alternatively, some microbes could be beneficial—either by outcompeting the inflammatory microbes or by changing how gluten is processed.

b. Genetic determinants of susceptibility to CeD and variation in tissue destruction. Genetics can be a powerful tool to identify new mechanisms associated with the development of VA. More than 200 loci are associated with CeD susceptibility, but mechanisms associated with these loci remain largely undetermined. We hypothesize that the functional impact of many of these GWAS variants may only be revealed in the context of active CeD. As a first step towards testing that hypothesis, we performed eQTL mapping on duodenal biopsies derived from 41 active CeD, 61 controls and 39 GFD patients for which we also had genotype data available for over 3 million single-nucleotide polymorphisms genome-wide. Across all three groups, >20% of genes expressed in duodenum samples were associated with at least 1 significant eQTL (referred to as eGenes), stressing the pervasive role of genetics to interindividual variation in gene expression levels. Interestingly, and consistent with our hypothesis, we found 388 eGenes that are only identifed among active CeD patients and not in the control and GFD group. These genetic associations would have been missed when only looking at healthy individuals, underlining the importance of studying regulatory processes in disease contexts to improve functional interpretation of as-yet unexplained GWAS loci and the mechanism of disease. Interestingly, we also found a large number of eGenes (n=580) only detectable in the GFD condition, supporting the notion that healing is associated with significant tissue remodeling that leads to sustained changes to the immune and regulatory landscape of the gut. In this RC2, we will significantly expand on these findings by characterizing eQTLs in the different cellular subsets identified by scRNA-seq. Specifically, the scRNA-seq data collected in the interventional cohorts will be paired with genotype data from the same individuals to generate the most comprehensive catalog to date of genetic variants that impact gene expression (eQTL) in IECs and the different subsets. This unique resource will directly inform on the mechanisms associated with known GWAS variants, and enable development of polygenic risk scores to stratify patients along a susceptibility continuum for developing severe VA.

De-challenge studies

a. Developing predictive models of tissue healing. We will perform ***de-challenge studies*** (**Fig. 3**), which will identify the interaction between the host response and microbial components and the pathways associated with variation in mucosal healing. As for the challenge studies, patients will serve as their own control as they will undergo 3 endoscopies over the course of the interventional study. The host and microbial analysis performed on samples obtained at the time of the initial endoscopy will allow to assess alterations present at the time of active CeD. The nature and intensity of these alterations can then be linked to the presence or absence of healing at 3 and 12 months after gluten exclusion. The 3-month time point will allow assessing

the early changes with gluten withdrawal. We expect that only 20% of patients will have healed by that time point. However, at this time, we will be able to detect changes in IECs that are secondary to loss of immune activation. We hypothesize that early changes in IECs may predict presence or absence of healing at 12 months (time of the third endoscopy). scRNA seq analysis of isolated IECs will allow to determine whether the transcriptional program of adaptive IECs (aVECs) and/or intestinal stem cells (ISCs) at the time of active CeD can be linked to the capacity or lack of capacity of the intestinal mucosa to heal. Intestinal organoids generated from IECs isolated from the 1st and last endoscopy will be biobanked and serve for future mechanistic studies. Furthermore, hypotheses on the mechanistic basis for IEC-immune-microbiome cross-talks preventing healing on a GFD will be generated as changes in microbiota and some transcriptional signatures will be associated and could predict the capacity of the tissue to heal.

b. Genetic determinants of susceptibility to CeD and variation in tissue healing. Genetic analyses and associations will be performed as in aim 1.2. to characterize genetic variants that impact gene expression of subsets of IECs modulating their lineage specification and regenerative capacity.

Research resources
This will be led by Dr. J. Johnson, co-investigator, and her Clinical Research Informatics (CRI) team, in collaboration with the RC2 PIs and co-PIs (Drs. Barreiro and Matthew), and Mine Cicek (biobank Mayo Clinic).

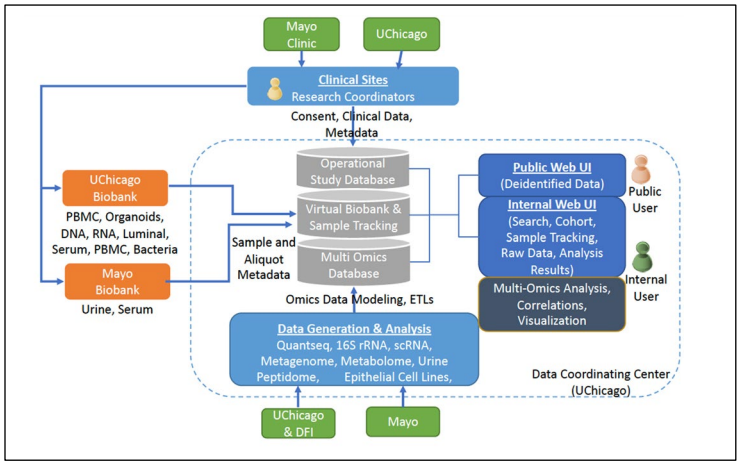


Fig. 4 An integrated, Web-based clinical research data and biobank management and informatics platform with data integrated from biobanks, clinical sites, sequencing cores, and omics analyses.

Description: The proposed RC2 will include coordinated collection of a diverse set of biosamples and datasets from Mayo Clinic, Caltech and U Chicago. It includes metadata from biobanks, clinical data, sequence data, and analysis results for a wide range of omic datatypes. To integrate such large and complex datasets and enable multi-omics analysis, we will build an integrated clinical research data management and informatics platform that will support harmonization, curation, dissemination, and analysis of the data collected at various levels (**Fig. 4**). This integrated platform will be developed and hosted at the data coordinating center at UChicago. It will include the following components: **1) Local Biobanks (Mayo Clinic & U Chicago) and Virtual Biobank:** While each of the two sites biobanks will have components of the RC2 samples, we will implement a *virtual biobank* in close collaboration with the site biobanks. This *virtual biobank* will centralize detailed metadata characterizing the biosamples

via an automated sync from each biobank. We will implement project specific identifiers that will be used as a cross-reference between various components and datatypes within the platform including the virtual biobank and the clinical study database described later**.** The *virtual biobank* will be a key component of the platform and will provide a powerful interface for an enhanced access to the metadata and sample tracking information; **2) Clinical Data:** Research coordinators from both clinical sites will upload all the relevant clinical data in a central study database that will be designed and implemented using REDCap; **3) Sequence & Analysis Results:** For all the various classes of sequencing performed during the RC2, we will develop a central repository of raw sequence data. We will implement analytical pipelines for all sequence data types and the results of analyses will be modeled and stored in a searchable *multi-omics database;* **4) WebUI:** The proposed platform will include two Web applications – a) Public Web application that will allow users to search and access all de-identified data, b) A robust Internal Web application with multi-faceted search capabilities, cohort creation capabilities, detailed sample tracking, and access to raw sequence data and analyses results; **5) Multi-omics analysis:** An innovative Web-based multi-omics module will support correlations between multi-omics datasets and clinical parameters of interest. It will include robust visualization of results for the results of the various omics data analyses.

8. Potential risks and benefits to subjects

a. Potential Risks:

The potential risks to study participants include those related to blood draws, upper endoscopy and/or those related to gluten challenge (for the “challenge” cohort).

Blood Draw Risks

Blood drawing could result in temporary slight discoloration of the skin after blood draws.

Endoscopy Risks

For participants undergoing endoscopy, the primary risks are related to unexpected sedation medication reactions (e.g., cardiopulmonary instability), aspiration, infection, perforation and bleeding. Based on ASGE guidelines, upper GI endoscopy carries low risk of adverse events. Large series report adverse event rates of 1 in 200 to 1 in 10,000 and mortality rates from none to 1 in 2000. Higher risk patients and higher risk procedures will be excluded in this study thereby minimizing risk.

Gluten Challenge Risks

Participants in the gluten challenge cohort could experience a number of GI or extra-intestinal symptoms with gluten ingestion. These symptoms could include abdominal pain, bloating, nausea, vomiting, diarrhea, constipation, headache, and fatigue. Based on our personal experience and a recent trial, CeD patients generally have mild to moderate symptoms on gluten with only a small number (~1%) requiring dose reduction or stopping the challenge. If subjects report symptoms as “severe” or “very severe” on the CDSD on any day, the research coordinator will alert the PI to review ongoing participation in the study vs. possible dose reduction.

Loss of Confidentiality:

As this study involves the use of subject identifiers and health information, there is a potential risk of loss of confidentiality.

b. Protection Against Risks:

Recruitment and Informed Consent
Prior to initiating the study, all subjects will provide written informed consent using forms approved by the Institutional Review Boards. The consent form becomes a permanent part of the medical record at each site participated. The consent form includes information relative to genetic studies and storage of genetic material for future research, as well as conforms with the high standards required for such studies. Since this genetic information is not yet pertinent to clinical practice, the information will not be included in the medical record; however, this information will be maintained in a coded fashion that excludes patient identifiers.

Loss of Confidentiality
There is the potential for loss of confidentiality. In order to minimize this risk, each subjects' intestinal samples will be assigned a random unique research ID number and barcode so that anonymity is preserved according to HIPPA regulations. Only the PI and the research team will have access to the file that links PHI to the study ID number and barcode. Any documents containing patient identifiers will be kept in locked files in the PI's office. Computer documents with PHI will be protected by passwords known only by the PI and research team

Blood Draw Risks
Blood will be drawn by study personnel trained in phlebotomy and using standard practices.

Endoscopy Risks
Endoscopy and tissue sampling will be performed by GI physicians experienced in endoscopic procedures, using standard endoscopic and tissue sampling methods. Inclusion and exclusion criteria insures that higher risk patients are excluded from this study.

Gluten Challenge Risks
The symptom severity tool will help guide whether a subject should be considered for study discontinuation. The CDSD is collected electronically using a secure RedCAP form that the study team will monitor regularly. Also subject symptoms will be monitored during all follow up phone calls. The study team will notify the PI regarding any subject who experiences severe symptoms. This will be assessed using the CDSD and any symptoms noted as "severe" or "very severe" will be discussed with the PI to determine ongoing participation in the study vs. possible dose reduction.

DSMP
A data and safety-monitoring plan (DSMP) has been established for this trial. Reporting mechanisms will be put in place for adverse events to the IRB and the NIH. An independent Data Safety and Monitoring Board will be appointed to oversee the trial as described in the application. This is described in section 9 below.

c. Potential Benefit to the Subjects and Others
The potential benefits to all participants include close assessment of diet and nutrition during the course of study. Participants in the de-challenge cohort will also have endoscopic assessments of mucosal healing and provide useful information for assessment of response to the gluten free diet.

d. Importance of the Knowledge to Be Gained
The indirect benefits are increased insight into the pathogenesis and heterogeneity of CeD. Specifically, this knowledge will improve our understanding of epithelial and microbial factors that either trigger CeD or promote patient susceptibility to this condition. In addition, the knowledge acquired through this study may allow for the future development of new risk biomarkers and interventions to prevent the development and/or treatment of CeD.

9. Monitoring of safety of subjects

Adverse Event Monitoring in Subjects:
Subjects will be monitored throughout the study for adverse events related to the gluten challenge, blood draws and endoscopy. The research coordinator will review daily CDSD for any subjects reports “severe” or “very severe” symptoms on any day during the gluten challenge. These will be discussed with the PI and determination made regarding ongoing participation vs. possible dose reduction in the amount of gluten. Any adverse events possibly related to blood draws or endoscopy will be discussed with the PI immediately as outlines below.

Adverse Event (AE) Reporting:
Any problematic or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s involvement in the research, whether or not considered related to participation in the research.

Serious Adverse Event (SAE):
Any adverse event that:

- Results in death
- Is life threatening, or places the subject at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Causes cancer
- Is an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

Unanticipated Problem:
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in nature, severity, or frequency, considering the research procedures described in this protocol and characteristics of the study population;
- Related or possibly related to participation in the research, meaning there is a reasonable possibility that the incident, experience, or outcome may have been caused by the study procedures and/or use of Montelukast;
- Is serious in nature or otherwise suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Severity
The severity of AEs will be assessed according to the following grading scale. Adverse Events will be classified according to severity, expectedness and potential relatedness to the study drug.

- Mild: Awareness of signs or symptoms, but easily tolerated and are of minor irritant type, causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
- Moderate: Events introduce a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- Severe: Events interrupt the subject's normal daily activities and generally require hospitalization.

Expectedness
AEs must be assessed as to whether they were expected to occur or unexpected, meaning not anticipated based on current knowledge of procedures and drugs involved in the research study. Categories are:

- Unexpected - nature or severity of the event is not consistent with known information about Montelukast or other study procedures.
- Expected - event is known to be associated with Montelukast or other study procedures.

Adverse Event Tracking
Adverse events will be gathered during each study and phone visit, as well as any spontaneous report occurring between scheduled study calls/visits. All adverse events will be evaluated and graded by the PI, and will be tracked in the study RedCap database.

Reporting Adverse Events
Adverse events will be reported to the IRB according to the IRB's reporting policy. AE data will be provided to the DSMB for analysis prior to all formal meetings, and more urgently in case of serious adverse events that are deemed related or possibly related to the study.

10. Data and Safety Monitoring

Multi-Center Coordination
The study teams at each site will conduct weekly research meetings to review recruitment, biospecimen collection, and any safety issues. A monthly meeting will be held with study personnel at University of Chicago and Mayo Clinic Rochester to review overall recruitment, biospecimen collection and any safety issues.

Data and Safety Monitoring Board (DSMB):
The Principal Investigator (PI) along with site PIs and study personnel will be responsible for ensuring participants' safety on a daily basis. The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to monitor participant safety, evaluate the progress of the study, to review procedures for maintaining the confidentiality of data, the quality of data collection, management, and analyses. The overall study PI is responsible for distributing adverse event reports and coordination across multiple sites. DSMB communications will be shared on a quarterly basis with all sites involved in the trial.

Frequency of Data and Safety Monitoring
The DSMB will meet twice annually by teleconference call to review study progress, data quality, and participants safety. Safety reports are sent to the site PI and PO at least twice a year and will include a detailed analysis of study progress, data and safety issues.

Data Analysis and Coordination
Data analysis and coordination will be performed by study personnel (but not the PI). All data from this trial will be directly available to the DSMB on request except raw data identifying individual participants. Since this is not an intervention trial, participants will not be notified of specific trial results. Any serious adverse events will be communicated with the subjects' health providers with appropriate information from the trial.

Content of Data and Safety Monitoring Report
The content of the data and safety monitoring report will include: study status, participant descriptive information, safety information, study quality.

DSMB Membership and Affiliation
The following individual(s) are proposed members of the DSMB. Should there be any questions regarding the independence of the DSMB, it will be addressed and corrected if necessary at that time.

Julian Solway, MD
Professor of Medicine, University of Chicago
Dr. Solway is Professor of Medicine, Dean for Translational Medicine and Director of the Institute for Translational Medicine. Dr. Solway is experienced in clinical and translational science and will provide important oversight regarding patient safety.

Christina Ciaccio, MD
Associate Professor of Medicine, University of Chicago
Dr. Ciaccio is the Section Head of Allergy and Immunology with expertise in allergic disorders including those related to food antigens. Dr. Ciaccio has been involved in a number of large drug trials in food allergy and will provide important oversight regarding patient safety.

Konstantinos A. Papadakis, MD
Professor of Medicine, Mayo Clinic Rochester
Dr. Papadakis is an adult gastroenterologist with expertise in intestinal disorders including celiac disease and inflammatory bowel diseases. Dr. Papadakis will provide important oversight related to gluten challenge and endoscopic safety.

Vandana Nehra, MD
Associate Professor of Medicine, Mayo Clinic Rochester
Dr. Nehra is an adult gastroenterologist with expertise in intestinal disorders including celiac disease. She will provide important oversight related to gluten challenge and endoscopic safety.

Conflict of Interest for DSMB
Each DSMB member will sign a **Conflict of Interest Statement** which includes current affiliations, if any, with pharmaceutical and biotechnology companies (e.g., stockholder, consultant), and any other relationship that could be perceived as a conflict of interest related to the study and / or associated with commercial interests pertinent to study objectives.

Protection of Confidentiality
Data will be presented in a blinded manner during the open sessions of the DSMB. At DSMB meetings, data and discussion are confidential. Participant identities will not be known to the DSMB members.

DSMB Responsibilities
The responsibilities of the DSMB include:
- Review the research protocol, informed consent documents and plans for data safety and monitoring;
- Recommend subject recruitment be initiated after receipt of a satisfactory protocol;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Review study performance, make recommendations and assist in the resolution of problems reported by the PI;
- Protect the safety of the study participants;
- Report to NIH on the safety and progress of the trial;
- Make recommendations to the NIH and the PI concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- Ensure the confidentiality of the study data and the results of monitoring; and,
- Assist the NIH by commenting on any problems with study conduct, enrollment, sample size, and/or data collection.

11. Payment to subjects:

Challenge Subjects:
Subjects will be compensated $300 after each endoscopy for a potential total of $900. If a subject screen fails based on lab results before the first endoscopic procedure, they will be paid $50 for the blood draw visit, and then withdrawn from study.

Dechallenge Subjects:
Subjects will be compensated $300 after each endoscopy for a potential total of $900.

Control Subjects:
Subjects will be compensated $300 for a single endoscopy. If a subject screen fails based on lab results before the first endoscopic procedure, they will be paid $50 for the blood draw visit, and then withdrawn from study.

12. Informed Consent
Subjects will undergo an initial screening research study visit. Consent will be obtained during this initial screening visit before any research-related procedures take place. Ample time will be given for consent review and questions.

13. Data and Sample Confidentiality and Sharing

Study data will be entered into a password protected REDCap database maintained on a HIPAA compliant, secure server hosted at the University of Chicago. All data will be entered and stored using the subjects unique study ID. The file that links the study ID to the subject's identity will be kept separately on password protected, HIPAA compliant, encrypted computers at the respective sites. The study data set will contain the dates of significant clinical events but will not contain any other subject identifiers.

Study samples will be labelled only with the subject's unique study ID and will not contain any other identifying information.

Plans for Sharing
The data and samples collected as part of this study will be banked and stored for future research. These material may be shared with other researchers with approval of the advisory committee (Drs. Jabri, Kupfer, Murray, Ismagilov, Abadie and Barreiro) and with proof of necessarily ethic review board approvals. Prioritization will be made depending on the specific request and availability of biobanked samples as well as the scientific merit. Data and samples will be shared using the subject's unique study ID only. Data may contain dates, but will not contain any other identifying information.

Data and samples will be also shared as required through the NIH. Data shared under this requirement be shared using the subject's unique study ID only. Data may contain dates, but will not contain any other identifying information.

14. Recruiting methods

Participants will be recruited primarily through the adult GI clinics at the University of Chicago and Mayo Clinic Rochester. The study team will also place an advertisement on the local institution's celiac center website (UofC - http://www.cureceliacdisease.org/). Additionally, subjects who have agreed to be contacted for future studies may be recruited through the celiac database at the University of Chicago (IRB #16103A - patients would be contacted via phone or email about the study). All patients will be screened for baseline eligibility by medical record review, and enrolled if they meet all inclusion and exclusion criteria.

Recruitment could include employees at the participating institutions. To minimize any risk of coercion, all recruitment and consenting activities with employees will be conducted by a study coordinator and will not involve any faculty from the study team.

15. Notification of subject's primary physician

The subject's primary care physician will be notified by electronic message through EPIC. If the primary care physician is outside UCM, a phone call and letter will be sent.

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Challenge study					
	Screening	Endoscopy visit (screening part 2) (2)	Day 0	Day 6	Week 6
Informed Consent	X				
Blood collection	X	X	X (1)	X	X
Gluten dispense (7, 8)			X	X	
Endoscopy		X		X	X
Biopsies		X		X	X
Luminal content		X		X	X
Vital signs	X				
Physical Exam	X				
PBMC		X		X	X
Urine		X	X	X	X
Saliva		X		X	X
Urine pregnancy test (3)		X		X	X
CDSD	X (4)	X	X	X	X
ICDSQ	X (5)	X	X	X	X
CDAT	X (6)	X	X	X	X

1. Serum collected 2 hour post challenge time
2. Patient moves to endoscopy portion of screening after celiac serology results are negative
3. Only applicable if child bearing, to be done prior to endoscopy
4. CDSD is a daily symptom survey. This will be performed once at time of screening, once at time of endoscopy and then daily beginning Day 0 through the end of the study period. This symptom diary will be administered electronically using RedCap.
5. ICDSQ is a weekly quality of life survey. This will be performed once at time of screening, once at time of first endoscopy and then weekly through the end of the study period.
6. CDAT is a measure of gluten free diet adherence. This will be performed at time of screening and time of first endoscopy and then at each endoscopy visit (day 6 and week 6).
7. Histology must be normal (VH:CD$\geq$ 2) to move forward to gluten challenge
8. Day 0 dispense gluten for day 0 to day 6. At Day 6 dispense 5 week gluten supply.

De-Challenge study			
	Screening/Day 0	3 months	12-13 months
Informed Consent	X		
Blood collection	X (7)	X	X (7)
Start gluten free diet (1)	X (2)		
Endoscopy	X (SOC)	X	X
Biopsies	X	X	X
Luminal content	X	X	X
PBMC	X	X	X
Urine	X	X	X
Saliva	X	X	X
Urine pregnancy test (3)	X (SOC)	X	X
IGFDQ (4)	X	X	X
ICDSQ (5)	X	X	X
CDSD (6)	X	X	X
CDAT (4)	X	X	X

1. Gluten free diet continues throughout study
2. Gluten free diet begins after histology is reviewed confirming diagnosis
3. Only applicable if child bearing, to be done prior to endoscopy
4. Study questionnaires to be completed prior to specimen collection and endoscopy
5. ICDSQ performed once at time of screening, once at time of first endoscopy and then weekly through the end of the study period.
6. CDSD is performed once at time of screening, once at time of first endoscopy and then daily through the end of the study period.
7. Baseline standard of care (or research if insurance doesn't cover or not done previously) labs: ferritin, 25 VD, zinc, copper, B12, folate, CBC, CMP, cholesterol; End of study standard of care labs (or research if insurance doesn't cover): ferritin, 25 VD, zinc, copper, B12, folate, CBC, CMP, cholesterol.

Control		
	Screening	Endoscopy visit
Informed Consent	X	
Blood collection	X (2)	X
Endoscopy		X
Biopsies		X
Luminal content		X
Physical Exam	X	
PBMC		X
Urine		X
Saliva		X
Pregnancy test (1)		X
CDSD (3)		X

1. Only applicable if child bearing, to be done prior to endoscopy
2. Patient moves to endoscopy portion of screening after HLA and celiac serology results confirmed
3. Symptom diary to be completed prior to specimen collection and endoscopy