SUPPLEMENTAL METHODS:

IgA+ and IgA- Bacteria Separation and Fecal IgA Flow Cytometry

Frozen human fecal samples were placed in Fast Prep Lysing Matrix D with ceramic beads (MP Biomedicals) and incubated in 1ml Phosphate Buffered Saline (PBS) per 100mg samples on ice for 5 min for hydrating, followed by homogenization using bead beating for 7 s (Minibeadbeater; Biospec) and then centrifuged 50g for 10 min at 4°C to remove large plaques. Fecal bacteria in the supernatants were collected (200 µl/sample) and washed three times with 500 µl PBS containing 1% (w/v) Bovine Serum Albumin (BSA, American Bioanalytical) and centrifuged for 5 min (6,000 x rpm, 4°C). A sample of this washed bacterial suspension (50 µl) was collected as the pre-sorting sample for 16S sequencing analysis. After washing, bacterial pellets were resuspended in 50 µl blocking buffer (PBS containing 1% (w/v) BSA and 20% Normal Mouse Serum, Jackson ImmunoResearch), incubated for 20 min on ice, and stained with 100 µl PE-conjugated mouse anti-human IgA (1:40; Miltenyi Biotec clone IS11-8E10) for 30 minutes on ice. Samples were subsequently washed 3 times with 500 µl BSA containing 1% (w/v) before flow cytometry analysis or cell separation. PE anti-human IgA stained bacteria were incubated with Anti-PE Magnetic Activated Cell Sorting (MACS) beads (Miltenyi Biotec) (1:5) for 30 minutes on ice and then separated by a custom magnetic plate for 10 minutes on ice. Fecal bacteria bound to the magnetic plate were collected as IgA+ samples for 16S sequencing analysis. Stained and MACS bead-bound bacteria unbound to magnet plate were collected (20~40 µl) and passed through MACS molecular columns (Miltenyi Biotec) (one sample/column) followed by flushing with 480 µl PBS containing 1% (w/v) BSA. The total pass-through (~500 µl) was loaded onto columns one more time. The columns were flushed with 500 µl PBS containing 1% (w/v) BSA. The total column pass-through (~1 ml) was saved as IgA- samples for 16S sequencing analysis.

16S rRNA Gene Sequencing and Statistical Analyses

All bacterial samples were suspended in 90 µls of MicroBead Lysis Solution with 10% RNAse-A and sonicated in a water bath at 50°C for 5 minutes. Samples were transferred to a plate containing 50 µls of Lysing Matrix B (MP Biomedicals) and homogenized by bead-beating for 5 minutes. After centrifugation (4122 x *g*, 4°C) for 6 minutes, the supernatant was transferred to 2 ml deep-well plates (Axygen Scientific). Bacterial DNA of the samples were extracted and purified using MagAttract Microbial kit (QIAGEN) following instruction provided by the manufacturer. PCR was performed to amplify the V4 region of 16S ribosomal RNA (33 cycles) in duplicate (3 µl purified DNA per reaction; Phusion DNA polymerase, New England Bioscience)[1]. After amplification, PCR products were then normalized with SequalPrep™ normalization plate kit (ThermoFisher Scientific) and pooled. The pooled library was calculated concentration by using NGS Library Quantification Complete kit (Roche 07960204001) and then loaded on a Miseq sequencer. Illumina Miseq Reagent Kit V2 (500 cycles) was used to generate 2x250bp paired-end reads. The raw reads were demultiplexed in Qiime1 (version 1.9). Reads were demultiplexed.

Bioinformatics analysis

Filtering and trimming of the bacterial 16s rRNA amplicon sequencing reads, and sample inference that turns amplicon sequences into an Operational Taxonomic Units (OTUs) table were performed by using *dada2*[2]. Exploratory and inferential analyses were performed by using *phyloseq*[3] and *vegan*[4], which includes Non-metric MultiDimenstional Scaling (NMDS) analysis,

Principle Components Analysis (PCA), alpha and beta diversity estimates, and taxa agglomerate. Differential OTU abundance was assessed by using *edgeR*[5]. P-values were corrected by using the Benjamini-Hochberg false discovery rate (FDR), and FDR < 0.25 was considered statistically significant[6]. The prediction of gene content and pathway abundance were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and *PICRUSt2*[7-9]. Differential KEGG pathway abundance was assessed by using *limma*[10]. The bar plots and box plots were made by using *ggplot2*[11], and heatmap by *pheatmap*[12]. The code for the bioinformatic analysis and statistical analysis of the data are available at https://github.com/jdreyf/celiac-gut-microbiome

Plasma Metabolomics Analysis.
Plasma samples for metabolomics analysis were prepared as previously described[13,14]. Metabolite extraction from plasma was achieved using a mixture of isopropanol, acetonitrile, and water at a ratio of 3:3:2 v/v. Extracts were divided into three parts: 75 uLs for gas chromatography combined with time-of-flight high-resolution mass spectrometry, 150 uLs for reverse-phase liquid chromatography coupled with high-resolution mass spectrometry, and 150 uLs for hydrophilic interaction chromatography with liquid chromatography and tandem mass-spectrometry, and analyzed as previously described[13,14]. We used the NEXERA XR UPLC system (Shimadzu, Columbia, MD, USA), coupled with the Triple Quad 5500 System (AB Sciex, Framingham, MA, USA) to perform hydrophilic interaction liquid chromatography analysis, NEXERA XR UPLC system (Shimadzu, Columbia, MD, USA), coupled with the Triple TOF 6500 System (AB Sciex, Framingham, MA, USA) to perform reverse-phase liquid chromatography analysis, and Agilent 7890B gas chromatograph (Agilent, Palo Alto, CA, USA) interfaced to a Time-of-Flight Pegasus HT Mass Spectrometer (Leco, St. Joseph, MI, USA). The GC system was fitted with a Gerstel temperature-programmed injector, cooled injection system (model CIS 4). An automated liner exchange (ALEX) (Gerstel, Muhlheim an der Ruhr, Germany) was used to eliminate cross-contamination from the sample matrix that was occurring between sample runs. Quality control was performed using metabolite standards mixture and pooled samples. A standard quality control sample containing a mixture of amino and organic acids was injected daily to monitor mass spectrometer response. A pooled quality control sample was obtained by taking an aliquot of the same volume of all samples from the study and injected daily with a batch of analyzed samples to determine the optimal dilution of the batch samples and validate metabolite identification and peak integration. Collected raw data were manually inspected, merged, inputted and normalized by the sample median.

Metabolite pathway analysis.
Metabolomic data was analyzed as previously described by Tolstikov et al.[15]. Identified metabolites were subjected to pathway analysis with MetaboAnalyst 4.0, using Metabolite Set Enrichment Analysis (MSEA) module which consists of an enrichment analysis relying on measured levels of metabolites and pathway topology and provides visualization of the identified metabolic pathways. Accession numbers of detected metabolites (HMDB, PubChem, and KEGG Identifiers) were generated, manually inspected, and utilized to map the canonical pathways. MSEA was used to interrogate functional relation, which describes the correlation between compound concentration profiles and clinical outcomes.

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