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Increased Intestinal Permeability Is Associated With Later Development of Crohn's Disease

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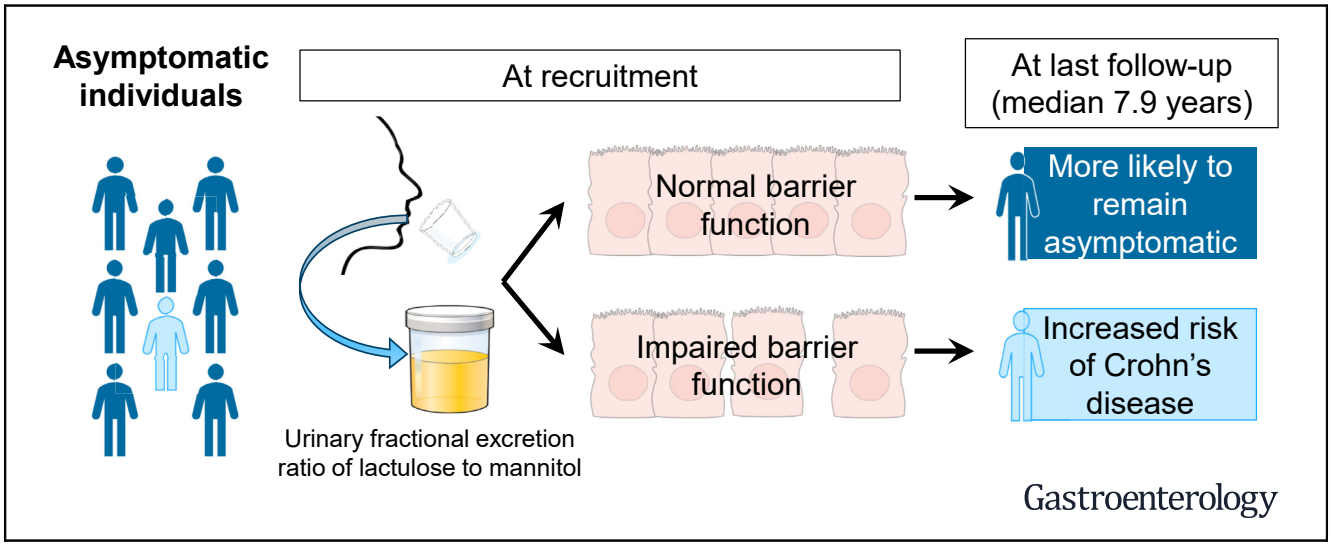

BACKGROUND & AIMS: Increased intestinal permeability has been associated with Crohn's disease (CD), but it is not clear whether it is a cause or result of the disease. We performed a prospective study to determine whether increased intestinal permeability is associated with future development of CD. **METHODS:** We assessed the intestinal permeability, measured by the urinary fractional excretion of lactulose-to-mannitol ratio (LMR) at recruitment in 1420 asymptomatic first-degree relatives (6–35 years old) of patients with CD (collected from 2008 through 2015). Participants were then followed up for a diagnosis of CD from 2008 to 2017, with a median follow-up time of 7.8 years. We analyzed data from 50 participants who developed CD after a median of 2.7 years during the study period, along with 1370 individuals who remained asymptomatic until October 2017. We used the Cox proportional hazards model to evaluate time-related risk of CD based on the baseline LMR. **RESULTS:** An abnormal LMR (>0.03) was associated with a diagnosis of CD during the follow-up period

(hazard ratio, 3.03; 95% CI, 1.64–5.63; $P = 3.97 \times 10^{-4}$). This association remained significant even when the test was performed more than 3 years before the diagnosis of CD (hazard ratio, 1.62; 95% CI, 1.051–2.50; $P = .029$). **CONCLUSIONS:** Increased intestinal permeability is associated with later development of CD; these findings support a model in which altered intestinal barrier function contributes to pathogenesis. Abnormal gut barrier function might serve as a biomarker for risk of CD onset.

Keywords: FDR Study; IBD; Gut Barrier; Crohn's Risk.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Increased intestinal permeability have been associated with Crohn's disease (CD), but it is not clear if these are risk factors for disease.

NEW FINDINGS

In an analysis of urine samples from asymptomatic first-degree relatives of CD, we found that abnormal intestinal permeability is a significant risk factor for CD onset, preceding diagnosis by as many as 3 years.

LIMITATIONS

It is possible that some participants had pre-symptomatic CD at the time of urine sample collection. However, the association between gut permeability and risk of CD diagnosis remained significant even after adjustments for potential confounding effect of subclinical inflammation.

IMPACT

Abnormal intestinal permeability appears to precede development of CD, and might be involved in pathogenesis. Strategies to restore gut barrier function might be developed to prevent CD in susceptible individuals.

The cause(s) of Crohn's disease (CD) remains unknown, although the current theory is that genetically susceptible individuals experience an environmental trigger(s), resulting in an inappropriate immune response, potentially against gut microbes. In this scenario, it has been suggested that gut barrier dysfunction serves as a risk factor of CD development. Although abnormal gut barrier has been measured in patients with established CD,[1–3] it is unclear if an abnormal barrier function exists before the development of CD. Previous literature has found that antimicrobial serology such as anti-saccharomyces cerevisiae antibodies (ASCA), anti-CBir1, and anti-OmpC could predict the onset of CD,[4] and a more recent study found that an additional 51 protein biomarkers were predictive of CD.[5] This suggests that in the face of a defect in the gut barrier, bacterial products may cross the gut barrier and induce an immune response before disease onset. Indeed, this is supported by a case report that found a defect of gut barrier function before the onset of CD.[6] However, to our knowledge, there have been no large-scale studies assessing if a gut barrier defect is present in indivdiduals who later develop CD. Many studies have found that abnormal intestinal permeability, measured by the urinary fractional excretion of lactulose-to-mannitol ratio (LMR), is more prevalent in patients with established CD than in healthy individuals.[7] However, these cross-sectional case-control studies cannot determine if the gut barrier defect precedes the onset of CD or if it is a consequence of the disease itself. Thus, we assessed if increased intestinal permeability is associated with the future risk of developing CD. We showed that abnormal LMR is associated with CD onset, and this association remained significant even when the test was performed more than 3 years before the development of CD.

Materials and Methods

Participant Recruitment

The study cohort comprised 1420 participants who were asymptomatic, first degree relatives (FDRs) of patients with CD for whom measures of baseline intestinal permeability were available among the Crohn's and Colitis Canada Genetic Environmental Microbial (CCC GEM) project cohort. Asymptomatic FDRs (siblings or offspring) of patients with CD, between 6 and 35 years of age, were eligible for recruitment into the CCC GEM project (Table 1). Before entry to the study, individuals were screened with a questionnaire to specifically exclude any history or symptoms of inflammatory bowel disease or any other gastrointestinal diseases. Specifically, the exclusion criteria for recruitment included unintentional weight loss in the last 3 months of more than 15% of baseline weight; having ever been diagnosed with any chronic or recurring gastrointestinal disease or bowel disease; abdominal pain that occurred more than once per week for longer than 3 months in the past year; diarrhea (>3 times per day) that has been occurring for more than 3 months in the last year; having blood in the stool with most stools; diagnosed with diabetes; diagnosed with celiac disease; diagnosed with irritable bowel syndrome; diagnosed with inflammatory bowel disease; and presenting significant symptoms of gastrointestinal disease. Demographic information and environmental risk data were recorded (Supplementary Notes 1 and 2), as previously described.[8] All participants and/or their guardians gave written informed consent to participate in the study.

The study was approved by the Mount Sinai Hospital Research Ethics Board (Toronto–managing center) and the local recruitment centers (see the list in the "Acknowledgments" section). All individuals were contacted every 6 months

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Abbreviations used in this paper: C-index, concordance index; CCC GEM, Crohn's and Colitis Canada Genetic Environmental Microbial; CD, Crohn's disease; CI, confidence interval; FDR, first degree relative; HR, hazard ratio; LMR, lactulose-to-mannitol ratio.

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Table 1. Demographic Data of the Cohort With Assessed Intestinal Permeability

Characteristics	Total cohort, n (%)	Cohort that developed CD, n (%)	Cohort that remain asymptomatic, n (%)	*P* value
Individuals	1420	50	1370	—
Sex: female, n (%)[a]	770 (54.2)	31 (62.0)	739 (53.9)	.79
Age at recruitment, *y*[a]				
Mean	19.3	17.4	19.4	.07
Median	19.0	16.0	19.0	
SD	7.8	7.6	7.8	
Relation to proband: offspring, n (%)[a,b]	395 (27.8)	12 (24.0)	383 (28.0)	.65
Current smoker, n (%)[a,d]	77 (5.4)	0 (0)	77 (5.6)	.08
Proband's age at recruitment, y[a,c]				
Mean, n	27.5	25.0	27.6	.17
Median, n	24.0	18.0	24.0	
SD, n	15.0	15.9	14.9	
Country of recruitment, n (%)[a]				
Canada	1379 (97.1)	45 (90)	1334 (97.4)	.01
United States	24 (1.7)	3 (6)	22 (1.6)	
Israel	17 (1.2)	2 (4)	14 (1.0)	
Time of follow-up, *y*, mean ± SD	7.06 ± 2.40	2.95 ± 2.14	7.21 ± 2.26	—
LMR, mean ± SD[a]	0.0196 ± 0.0173	0.029 ± 0.035	0.0194 ± 0.0159	.015

SD, standard deviation.
[a]*P* values are obtained using the Mann-Whitney *U* test or Fisher exact test.
[b]Individuals were either offspring or sibling.
[c]No data were obtained for 6 individuals.
[d]Smokers are defined as individuals smoking at least 1 cigarette a day. No data were obtained for 687 children or adult individuals.

by phone (Supplementary Notes 1 and 2). When an individual disclosed that he or she was diagnosed with CD, this was confirmed by the treating physician (Supplementary Note 3), and the site director reviewed any official documentation. The analysis described here comprised 50 individuals who developed CD during the study period, along with 1370 individuals who remained asymptomatic to date (as of October 2017) (Table 1, Supplementary Table 1, and Supplementary Figure 1). The study cohort described here is a subset of the CCC-GEM Project cohort, to include 1196 individuals with urine sample available and LMR analyzed (including 35 pre-CD individuals as of January 2014). To increase the power to detect significant associations, we included the initial batch of 1196 samples available as of 2014 and added a second batch of 244 samples measured later in 2015. The second batch of data included samples from 15 individuals who later on developed CD and 229 individuals who remained asymptomatic controls (Supplementary Figure 1).

Assessment of Intestinal Barrier

Intestinal barrier permeability was measured in vivo by the ratio of the fractional excretion of lactulose to mannitol, as previously described[7,9–11] (Supplementary Figure 2*A–D*). In brief, participants were required to refrain from ingestion of alcohol, aspirin, and other nonsteroidal anti-inflammatory agents for at least 5 days before the probe administration. A standard solution of lactulose (5 g), mannitol (2 g), sucrose (100g), and flavored drink crystals (1.5 g) in 500 mL of tap water was ingested before bed. Urine was collected the following morning in a container (with 5 mL of thymol solution) and returned to the study center, where the total urine volume was measured, and 5-mL aliquots were frozen at –80°C. The concentrations of lactulose, mannitol, and sucrose were measured by high-pressure liquid chromatography.[7,11] Samples were run on a Dionex ICS-3000 system with autosampler by using high-performance anion exchange chromatography with pulsed amperometric detection. A linear standard curve measures mannitol between 0.0625 and 4 mg/mL and lactulose between 0.00625 and 0.4 mg/mL; 200 mg/mL cellobiose is used as an internal control. To prevent buildup of deposits on the electrode, which will gradually reduce the sensitivity of detection, a standard blank solution is inserted between every sample, and calibration is reperformed. We found that the separation and quantification were highly reproducible. The retention time for mannitol was 20.7 ± 0.07 minutes, and for lactulose it was 27.3 ± 0.2 minutes. Samples measured at different points during the same run have 100% reproducibility. We have run validation assays and found that the same samples run 4 days and 2 years apart had mean LMR differences of 0.001 ± 0.001, whereas the same samples run 5 years apart had mean LMR differences of 0.002 ± 0.002. The fractional excretion of lactulose and mannitol was calculated as the ratio of the total urinary excretion of the respective saccharide

probe to the total oral dose of the probe. For each participant, the LMR was calculated as the fractional excretion of lactulose divided by that of mannitol (Supplementary Figures 2 and 3). In addition to LMR, we assessed the fractional excretion of each of the individual probes, including lactulose, mannitol, and sucrose (Supplementary Figure 2).

Assessment of Subclinical Inflammation

Participants are instructed to collect their baseline stool samples using the provided stool commode from Thermo Fisher Scientific (Waltham, MA). A polypropylene specimen collection container (Starplex Scientific Inc, Etobicoke, ON) is used to take an aliquot of stool from the commode. The stool sample is then immediately placed into the participant's home freezer. To prevent freeze thawing, the home-frozen stool sample is transferred by the participant frozen using icepacks to the recruitment center, where it is stored in monitored −80°C freezers. Periodically, recruitment sites will batch ship the accumulated stool samples to the GEM Project's core facility. To ensure that samples remain frozen during the shipping process, these shipments use World Courier Cold Chain Logistics and are shipped on dry ice with temperature probes to ensure that the samples remain frozen. Shipments are topped up with dry ice as needed to ensure the samples remain frozen. Once received at the core facility, samples are cataloged and immediately placed in −80°C freezers that are on emergency back-up power and dual temperature monitoring systems. Fecal calprotectin concentration was measured by the Bühlmann fCAL enzyme-linked immunosorbent assay test (Schöonrenbuch, Switzerland) following the manufacturer's protocol, and the average of the duplicate values was used to define the calprotectin concentration. We selected a working range of 30–1800 μg/g of fecal calprotectin. Briefly, 50–100-mg stool samples are homogenized in the extraction buffer by vortexing for 30 minutes with appropriate dilution factors. The homogenate was centrifuged for 5 minutes, and the supernatant was collected and centrifuged again for 5 minutes. The supernatant was collected and used for the enzyme-linked immunosorbent assay, as per the manufacturer's protocol. The absorbance at 450 nm was measured on a microtiter plate reader. Fecal calprotectin concentration was dichotomized with a threshold of 100 μg/g to determine the presence and absence of subclinical inflammation.[12,13] A total of 297 (21.9%) participants had a fecal calprotectin level above 100 μg/g.

Statistical Analysis

To evaluate the time-related risk of developing CD in our cohort based on the baseline LMR, we performed a survival analysis using the Cox proportional hazards model. The individuals who were lost to follow-up were right-censored as being healthy at the latest health review that the participant agreed to perform. LMR was assessed both as a continuous variable and a categorical variable. Based on previous case-control studies that investigated intestinal permeability in patients with CD and healthy control individuals,[9,11,14–17] abnormality in the gut barrier can be defined with a stringent threshold (LMR $>$ 0.03) or a more permissive threshold (LMR $>$ 0.025). Each participant's urine excretion values of saccharides were assessed as continuous variables. The hazard ratio for continuous variables was presented as the relative risk for every 1/15-unit increase for LMR (continuous LMR), every 0.1-unit increase for fractionally excreted mannitol, every 0.01-unit increase for fractionally excreted lactulose, and every 0.001-unit increase for fractional excretion of sucrose. Age, sex, and family cluster were included in the model to account for potential confounding effects. In the subset of the cohort that had both stool and urine samples available, the fecal calprotectin variable was included to adjust for the presence or absence of subclinical inflammation.

Finally, to define the optimal LMR threshold taking time to disease into account, we used a multivariate categorization of LMR based on the concordance index, adjusting for age and sex (R 3.0.1, CatPredi).[18] P values lower than .05 were regarded as statistically significant. R software (R Foundation for Statistical Computing, Vienna, Austria), version 3.5.3, was used for statistical analysis.

URL

Details of the project can be found at http://www.gemproject.ca/data-access/.

Results

Baseline Characteristics of Participants

The study cohort comprised 1420 individuals recruited between 2008 and 2017 as part of the CCC GEM project (Table 1). The median age was 19 ± 7.8 years, 42.2% of participants were female, and the mean time between recruitment and follow-up was 7.06 ± 2.4 years. We did not find major demographic differences between participants who remained asymptomatic and participants who later developed CD (Table 1).

Previous case-control studies comparing CD and healthy control individuals have suggested an abnormal LMR,[9,11,14–17] using a stringent threshold of LMR higher than 0.03 or a more permissive threshold of LMR higher than 0.025. Using these thresholds, the study cohort comprised 165 individuals (11.6%) with an LMR higher than 0.03 and 274 individuals (19.3%) with an LMR higher than 0.025 at recruitment.

Abnormal Permeability Precedes Crohn's Disease Onset

We found that baseline LMR measures at recruitment of individuals who later developed CD were significantly higher than those of individuals who remained asymptomatic ($P < .015$; Mann-Whitney U test) (Supplementary Figure 3). Taking the observed follow-up time into account and assessing LMR as a continuous variable, a multivariate Cox proportional hazards analysis adjusting for age, sex, and family cluster showed that an increased LMR at recruitment was significantly associated with an increased risk of developing CD ($P = 7.6 \times 10^{-4}$; hazard ratio [HR], 1.92; 95% confidence interval [CI], 1.31–2.80). Age was associated with CD onset ($P = .046$; HR, 0.962; 95% CI, 1.04–0.962) but not sex (male vs female) ($P = .12$; HR, 0.63; 95% CI, 1.57–0.36). Of note, among the pre-CD samples, the LMR measurement tends to be higher when measured

closer to disease onset (Supplementary Figure 4). When the LMR was analyzed as a dichotomous variable with abnormal intestinal permeability, defined as LMR higher than 0.025, we obtained a P value of .011, HR of 2.12, and 95% CI of 1.19–3.79 (Supplementary Figure 5). Using a more stringent threshold to define abnormal intestinal permeability as LMR higher than 0.03, our ability to detect risk was even greater: $P = 3.97 \times 10^{-4}$; HR, 3.03; 95% CI, 1.64–5.61 (Figure 1). We further investigated each probe separately (ie, lactulose, mannitol, and sucrose) because they reflect different pathways and locations of gut barrier and could help in characterizing the gut barrier dysfunction.[19–21] Only the excreted fraction of mannitol was significantly associated with development of CD, whereas the others were nonsignificant (Supplementary Table 2).

Abnormal Permeability Is Associated With Future Crohn's Disease Onset Many Years Before Diagnosis

To investigate whether the relative risk of CD onset for abnormal LMR (hazard ratio) is influenced by the length of observation time before disease onset, we split the data set into participants who were followed for more than 3 years (including 1277 asymptomatic individuals, of whom 23 developed CD) and less than 3 years (including 143 asymptomatic individuals, of whom 27 developed CD). We chose 3 years because it was approximate to the median observation time (2.95 ± 2.14 years) of our cohort. We found that LMR was significantly associated with disease onset regardless of whether the follow-up duration was within or more than 3 years (Table 2). Specifically, we found that in individuals with an LMR measured at less than 3 years of follow-up, LMR was a significant predictor of CD onset ($P = 6.03 \times 10^{-4}$; HR, 9.3; 95% CI, 2.6–33.2). In comparison, when LMR was measured more than 3 years before disease onset, we obtained a significant yet smaller HR ($P = .029$; HR, 1.62; 95% CI, 1.051–2.50).

Gut Permeability as a Preclinical Biomarker of Crohn's Disease Onset

We further investigated the performance of LMR as a preclinical biomarker of future risk of disease using the concordance index (C-index) in the cohort by varying the threshold of LMR. We used the C-index to quantify the discriminative ability of LMR to predict the time to a censored event. We found that the C-index was 59.8%, with an optimal threshold of LMR >0.035, to classify asymptomatic individuals who went on to develop CD vs those more likely to remain healthy.

We also assessed whether abnormal gut permeability as defined by an LMR of >0.035 could be confounded by the presence or absence of subclinical gut inflammation, as reflected by a fecal calprotectin concentration higher than 100 μg/g. A total of 1355 participants had both LMR and fecal calprotectin data available at recruitment, including 44 pre-CD individuals. Notably, LMR and fecal calprotectin showed a weak correlation at baseline ($R = .069$; $P = .012$, Spearman correlation) (Supplementary Figure 6). Even after adjustment for fecal calprotectin higher than 100 μg/g, individuals with an LMR higher than 0.035 were at increased risk of developing CD ($P = .041$; HR, 2.23; 95% CI, 1.03–4.81) (Supplementary Table 1). Hence, the permeability defect in a subset of this cohort is likely reflective of an early functional abnormality that precedes the clinical onset of CD and not simply a reflection of subclinical inflammation.

Discussion

The causes of CD remain unknown, and case-control studies have identified that gut barrier function is abnormal in patients with CD compared to control individuals. We found that gut permeability was associated with the onset of CD. To our knowledge, this is the first study of a large cohort to prospectively show that increased intestinal permeability in asymptomatic individuals is significantly associated with the future risk of developing CD.[6] In addition, hazard ratios reported here for the abnormal LMR suggest that elevated intestinal permeability is an important risk factor in CD etiology. It is unknown whether the abnormality of the gut barrier is a reflection of the luminal environment, the consequence of a specific insult, or an intrinsic barrier defect. Our recent analysis of the genome-wide association of LMR showed that host genetics provide only a small contribution to an abnormal LMR,[10] suggesting that abnormal LMR may be more likely a reflection of an environmental trigger or insult.

We showed that LMR is capable of classifying individuals who are likely to develop CD with a C-index of 59.8%, with an optimal LMR threshold of 0.035. The performance of LMR was in the same range as those of pre-CD serology biomarkers represented by the area under the receiver operating characteristic curves, such as perinuclear antineutrophil cytoplasmic antibody, ASCA IgA, ASCA IgG, and *Escherichia coli* outer membrane porin C (OmpC), with area under the receiver operating characteristic curves of 53.4%, 58.2%, 58.1%, 55.8%, respectively, in previous studies.[4,22] This LMR threshold was similar to those generally defined in the literature for LMR in patients with established CD or irritable bowel syndrome.[23] Even after using this threshold, it is noteworthy that 88 of 101 (87%) individuals with abnormal intestinal permeability remained asymptomatic to date over the course of the study, with a mean follow-up time of 6.7 ± 2.04 years. In fact, the cumulative incidences of CD at 1, 3, and 5 years among the group with LMR of >0.035 were 4.0%, 8.0%, and 11.5%, respectively, compared to 0.5%, 1.5%, 2.4% in the group with LMR of <0.035. The possibility of remaining refractory to disease or being protected despite the presence of abnormal intestinal permeability is supported by a mouse experimental study for which a transient defect in gut barrier function, induced by a single exposure of ethanol and AT1002 (a Vibrio cholerae zonula occludens toxin), was protective against trinitrobenzene sulfonic acid–induced colitis.[24] This may indicate that the effect of impaired gut permeability on immune activation and intestinal inflammation may indeed be dependent on the context in which those events occur or, alternatively, dependent on the presence or absence of some

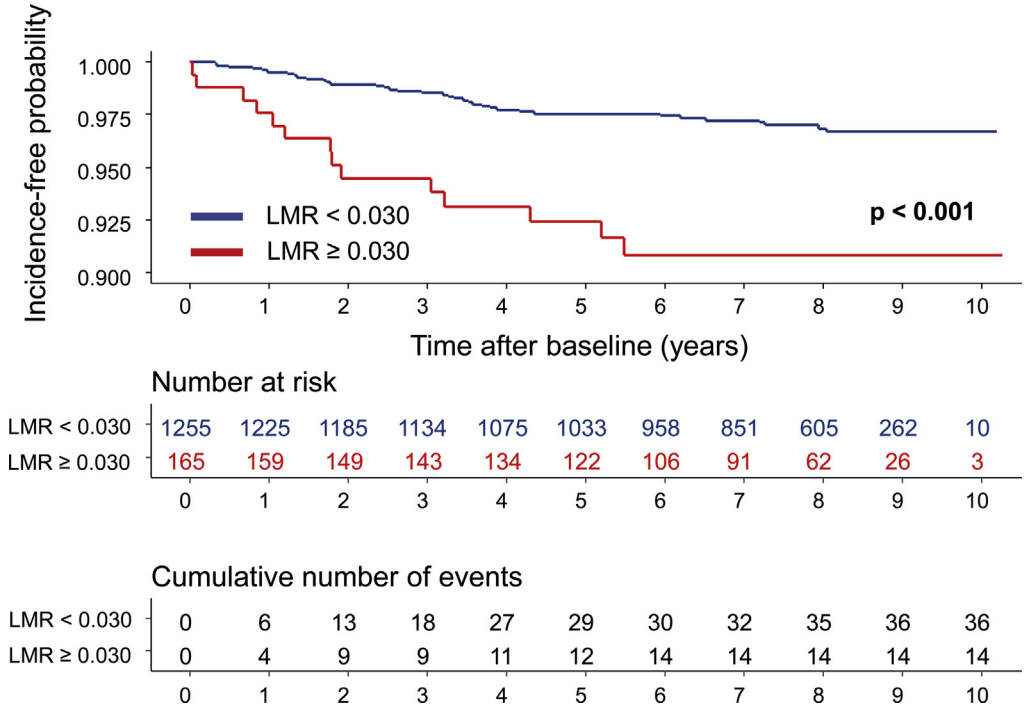

Figure 1. Increased intestinal permeability is a risk factor associated with CD onset. The *x*-axis represents the time (in years) between the initial measurement of intestinal permeability as measured by the LMR and the last follow-up of the participant. The *y*-axis represents the incidence-free probability of developing CD. The blue line represents the results for a normal permeability with a LMR lower than 0.030. The red line represents individuals with an abnormal permeability with a LMR higher than 0.030. The Kaplan-Meier curve was plotted based on the univariable model. The *P* value was based on the log-rank test.

particular environmental exposure.[25] At this time, it remains unclear if increased intestinal permeability may have a persistent effect on the host homeostasis and if this would potentially lead to the development of disease in some. However, mechanistic studies previously showed that reducing small intestinal permeability attenuates colitis in the *IL10* gene–deficient mouse experiment and in the T-cell transfer model.[26–28] Also, a decreased epithelial intestinal surface area in the small intestine represented by mannitol[29] seemed to be associated with CD onset; however, this should be interpreted with caution, because other factors, such as incomplete ingestion of the solution and differences in gut transit time, can affect the results. Instead, the use of LMR adjusts for these potential confounding issues.[2]

We acknowledge that it is unclear if some of the participants may have had evidence of subclinical disease despite having no symptoms at the time of recruitment. It remains possible that some individuals may have early presymptomatic CD; however, this proportion is likely small. A study of 147 asymptomatic FDRs of patients with CD who underwent video capsule endoscopy showed that only 1 individual had marked inflammation typical of CD.[30] Indeed, previous studies that followed asymptomatic patients with isolated terminal ileitis that was found in screening colonoscopies showed that fewer than 1.1% to 4.6% develop CD during a median follow-up of 29 to 54 months.[31,32] Follow-up data on asymptomatic FDRs with mild inflammation are still lacking. Nevertheless, the association of LMR and future development of CD was consistently significant in the subgroup of participants followed up for more than 6, 12, and 36 months before the diagnosis date. This indicates that gut barrier dysfunction is present long before diagnosis and is associated with the risk of developing overt CD (Supplementary Table 3). These results suggest that abnormal permeability is an early preclinical

Table 2. HR of Developing CD Based on LMR Measurement Less or More Than 3 Years Before Diagnosis

Traits	Variables	HR	95% CI	*P* value
LMR[a] less than or equal to 3 years (116 HC, 27 pre-CD)	LMR	9.3	2.60–33.22	.0006
	LMR ≥ 0.030	3.58	1.42–8.98	.0067
	LMR ≥ 0.025	2.97	1.25–7.02	.0133
LMR more than 3 years (1254 HC, 23 pre-CD)	LMR	1.62	1.05–2.50	.0290
	LMR ≥ 0.030	2.14	0.80–5.70	.1289
	LMR ≥ 0.025	1.44	0.57–3.60	.4404

HC, healthy control.
[a]The LMR HR is presented for each increase of 1/15 unit of LMR. The coefficients presented are adjusted for age, sex, and family cluster.

event that precedes the symptomatic onset of CD by several years and, potentially, a critical component to the pathogenesis of CD. Furthermore, abnormal permeability was associated with future onset of CD, even after adjusting for the presence or absence of subclinical inflammation, suggesting that the permeability defect is not simply a reflection of subclinical inflammation but, rather, an independent preclinical event in a subset of individuals.

In conclusion, we found that increased intestinal permeability as measured by the LMR is a significant risk factor that precedes CD onset by as many as 3 years. To our knowledge, this prospective study is the first to show that abnormal intestinal permeability is a significant predisease risk factor of CD onset that precedes disease development by more than 3 years. In spite of many open questions, intestinal permeability is becoming an area of growing interest, both in basic science and translational research, because it may offer a new target for disease prevention and therapy.[33] There are currently no available therapies to directly target and restore the intestinal barrier approved by the US Food and Drug Administration for this purpose.[2] Our results suggest that studies are required to discover new drugs to potentially prevent disease onset by promoting and maintaining a healthy gut barrier, especially considering that CD affects more than 800,000 individuals in North America and 1.6 million individuals in Europe, along with increasing numbers in many areas of the world.[34,35] The absence of a cure for CD emphasizes the importance of this study because the identification of early preclinical markers of disease could lead us toward prevention strategies.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2020.08.005.

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Data availability: Requests for raw and analyzed data should follow the instructions given at http://www.gemproject.ca/data-access/. All submissions will be reviewed by the GEM Project Operating Committee to ensure that the requested samples/data will not interfere in any way with the intended GEM Project analysis of the nested cohort as per the original GEM Project Study Design and is not a duplication of analysis already ongoing. Those proposals meeting this evaluation will be distributed to all members of the GEM Project Steering Committee for review and open discussion. This review will focus on the global scientific merit of the proposal. This review will assess the basic scientific merit and the availability of requested samples and data, ensuring there is no compromise of the original intent of the GEM project. It would be of value to contact a member of the Steering Committee who could help sponsor your application. Those projects achieving a majority vote of approval at the GEM Project Steering Committee will be informed that the GEM Project will provide a letter of support stating that the requested samples or data will be made available to the applicants once the applicant receives funding from a granting agency that applies an independent peer review process to the proposal. The criteria to be used for review of all submissions will include the "scientific relevance" of the proposal and the judged availability of biological material requested. The budget to be requested from a funding agency must allow for any expenses in processing samples or in setting up the appropriate queries of the database. The intent is to allow sufficient time for applicants to consider submission for funding opportunities.

CRediT Authorship Contributions
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Conflicts of interest
The authors disclose no conflicts.

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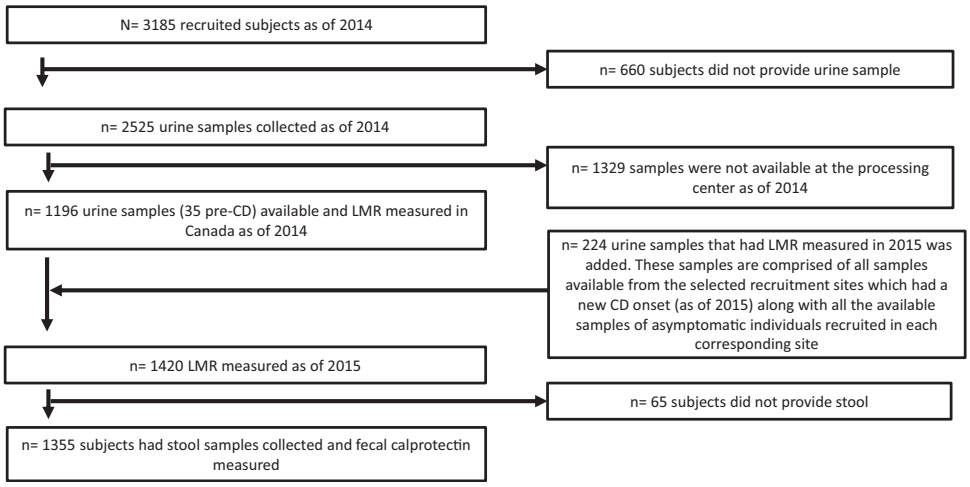
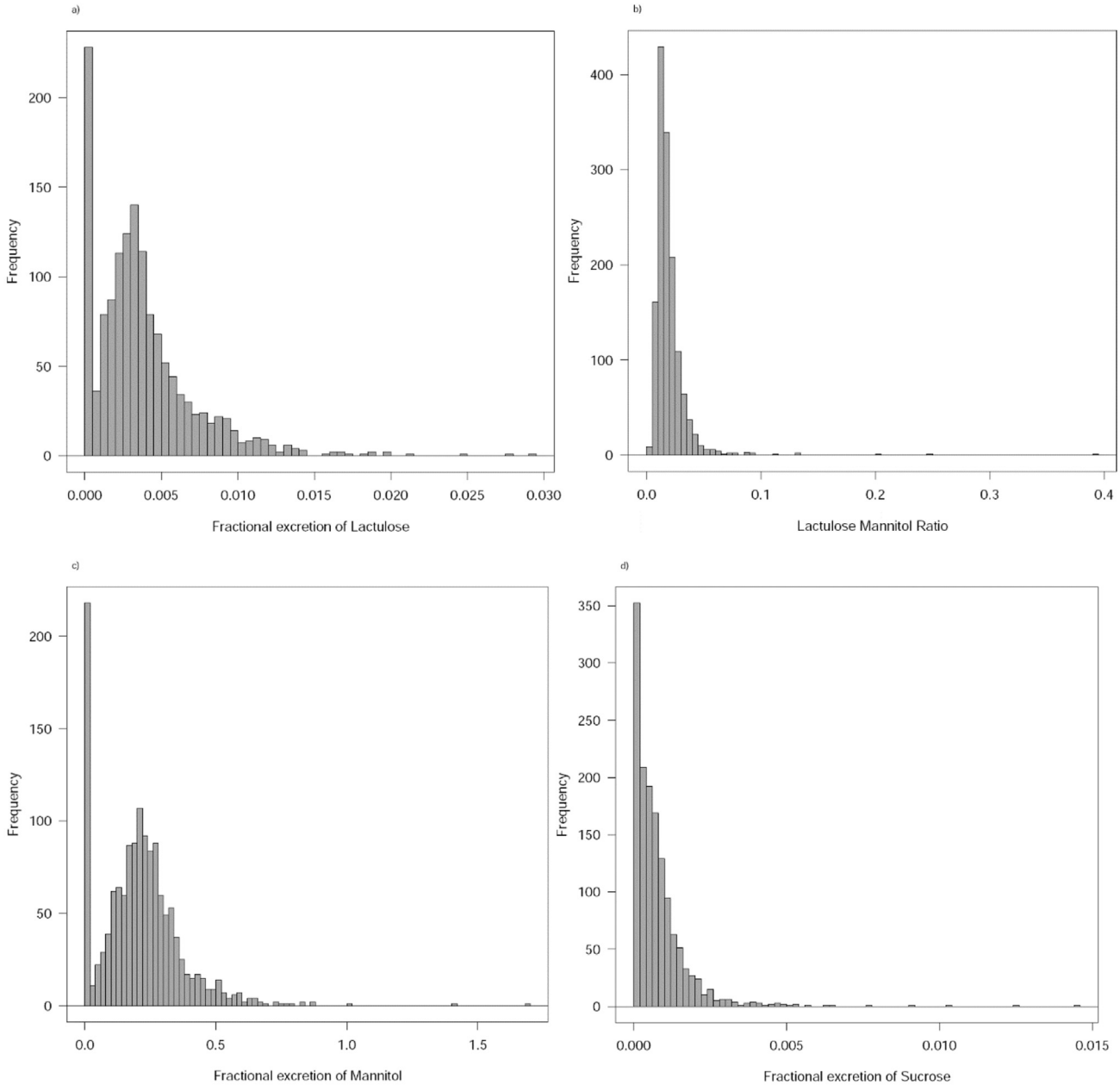
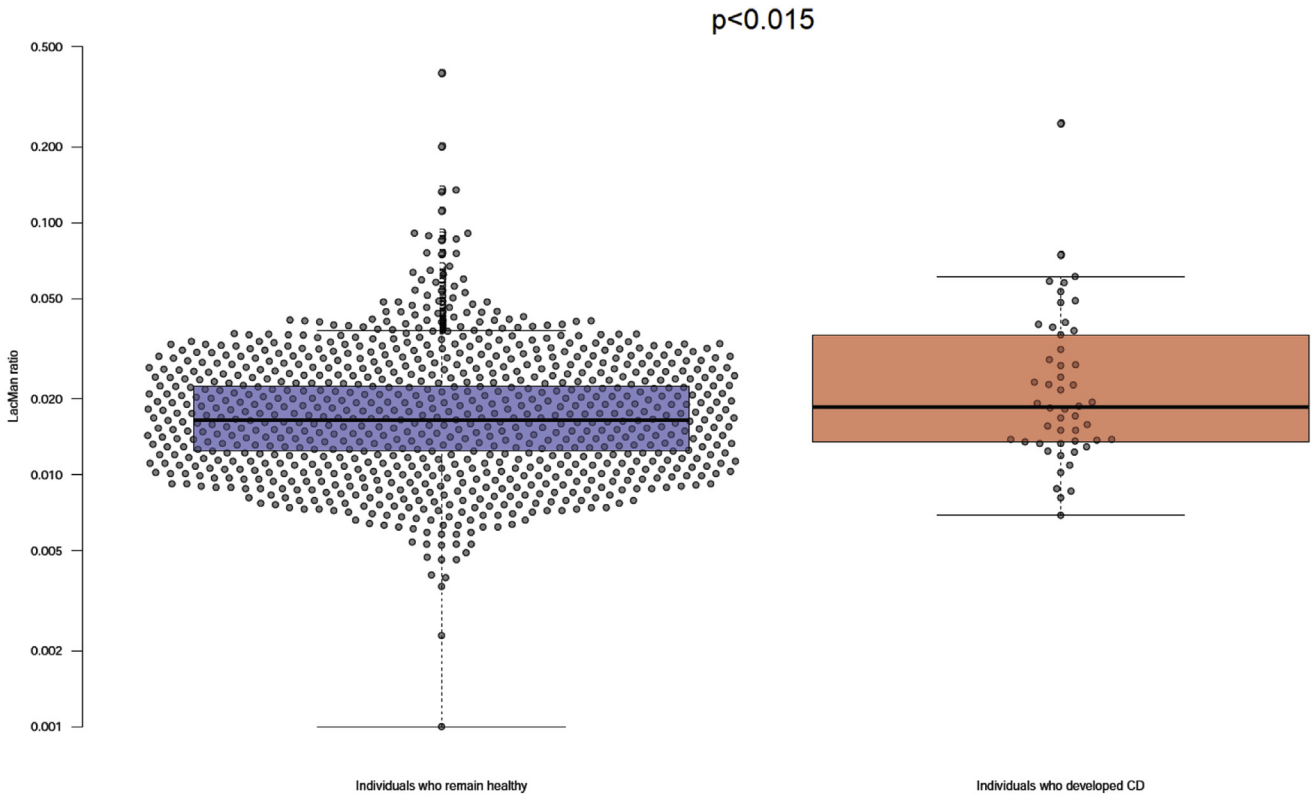


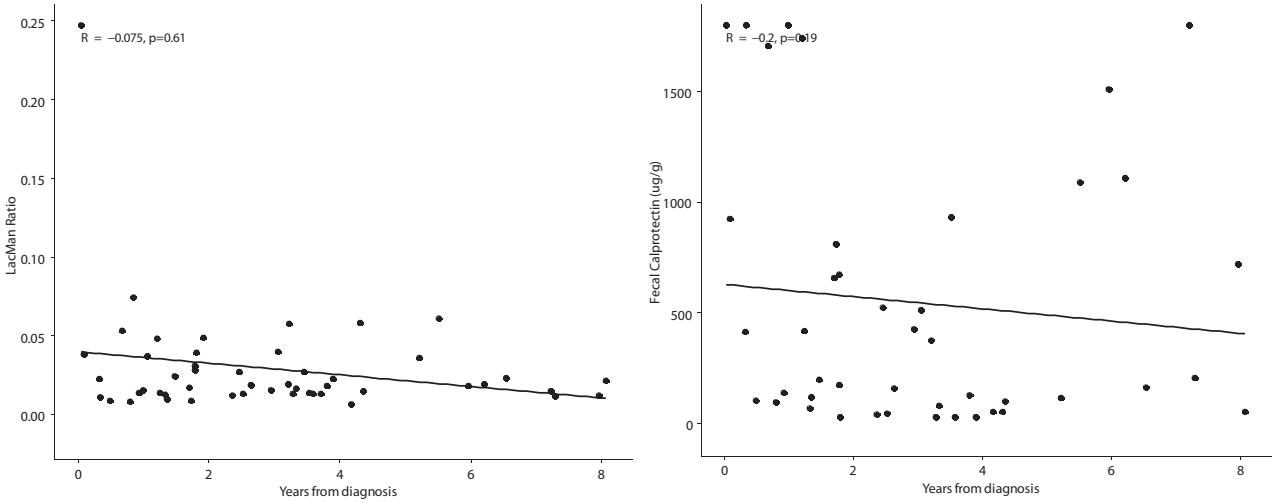
Supplementary Figure 1. Flow chart of participants excluded from the analysis. A total of n = 236 technical duplicates was removed as part of the quality control.



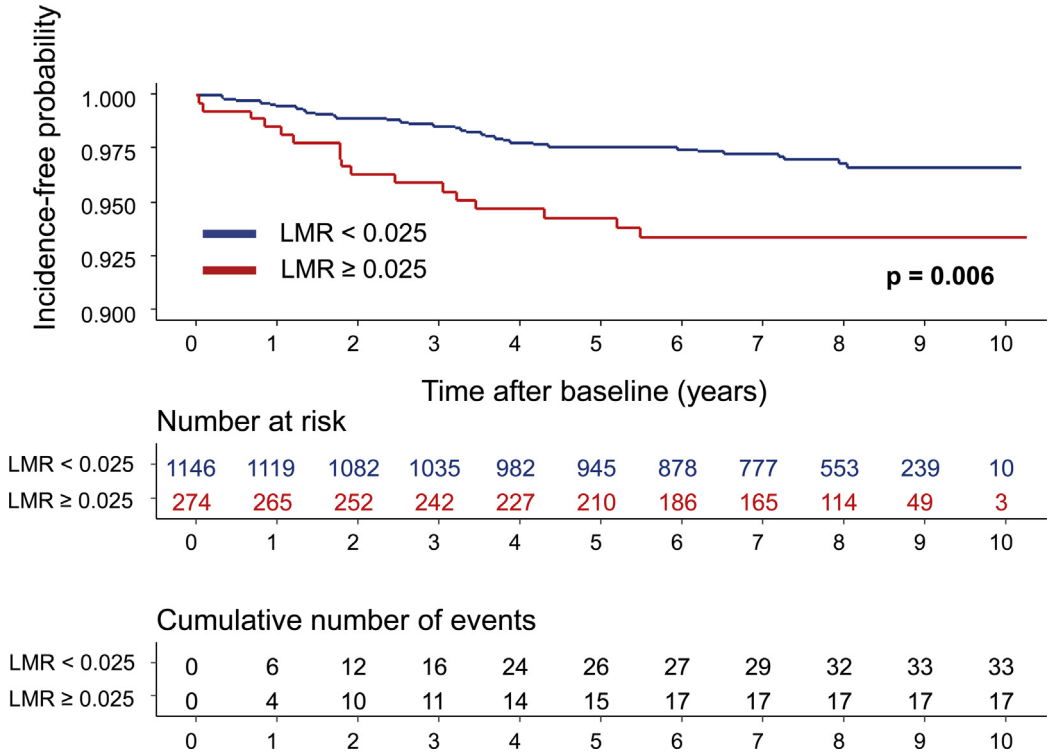
Supplementary Figure 2. (*A–D*) Distribution of LMR and the fractional excretion of lactulose, mannitol, and sucrose in the entire cohort.



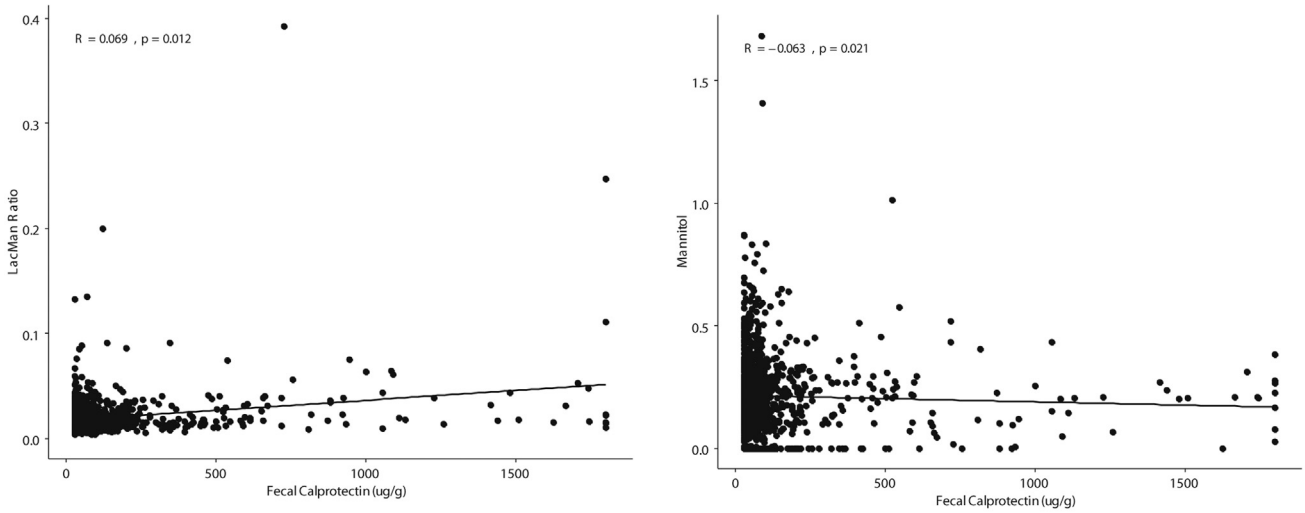
Supplementary Figure 3. The LMR ratio is increased in individuals who later develop CD compared to individuals who remained asymptomatic. The *x*-axis plots the corresponding individuals who remained healthy (*blue*) and those who developed disease (*red*). The *y*-axis represents the LMR on a log scale. The lines in the boxplot represent the first, second, and third quartiles. Circles represent the LMR from an individual. The *P* value was based on the Mann-Whitney *U* test. LacMan, lactulose to mannitol.



Supplementary Figure 4. Correlation between LMR or FCP with follow-up years before diagnosis. **R* represents the Spearman correlation coefficient, and the *P* value is for the statistical test to reject the hypothesis of lack of correlation ($R = 0$).



Supplementary Figure 5. Dichotomized intestinal permeability higher than 0.025 is a risk factor associated with CD onset. The *x*-axis represents the time (in years) between the initial measurement of intestinal permeability, as measured by the LMR, and the last follow-up of the subject. The *y*-axis represents the incidence-free probability of developing CD. The blue line represents the results for a normal permeability with an LMR lower than 0.025. The red line represents individuals with an abnormal permeability with an LMR higher than 0.025. The Kaplan-Meier curve was plotted based on the univariable model. The *P* value was based on the log-rank test.



Supplementary Figure 6. Correlation between LMR or mannitol and FCP in the entire cohort. **R* represents the Spearman correlation coefficient, and the *P* value is for the statistical test to reject the hypothesis of lack of correlation ($R = 0$).

Supplementary Table 1. HR of the LMR and covariates in the cohort that had fecal calprotectin and LMR data available

Variable	Adjusted HR[a]	95% CI	*P* value
LMR > 0.035	2.23	1.03–4.81	.041
FCP > 100	7.76	3.99–15.11	<.001
Age[a]	0.97	0.93–1.02	.231
Male vs female	0.65	0.35–1.18	.155

[a]The age HR is presented for each increase of 1 year of age.

Supplementary Table 2. HR of Individual Lactulose, Mannitol, and Sucrose Probes

Traits	HR	95% CI	*P* value
Lactulose[a]	0.76	0.14–41.15	.75
Mannitol[b]	0.68	0.47–0.99	.043
Sucrose[c]	0.91	0.50–1.63	.74

[a]The HR for the fractional excretion of lactulose is represented for each increase of 0.01 unit of fractionally excreted lactulose.
[b]The HR for the fractional excretion of mannitol is represented for each increase of 0.1 unit of fractionally excreted mannitol.
[c]The HR for the fractional excretion of sucrose is represented for each increase of 0.001 unit of fractionally excreted sucrose.

Supplementary Table 3. HR of the LMR in Subgroups Based on Follow-Up Time

Population	Time from baseline to diagnosis/ last follow-up date, *mo*	Adjusted HR[a]	95% CI	*P* value
45 pre-CD/1358 HC	>6	1.70	1.20–2.40	2.8×10^{-3}
40 pre-CD/1342 HC	>12	1.62	1.13–2.31	8.2×10^{-3}
23 pre-CD/1254 HC	>36	1.62	1.05–2.50	2.9×10^{-2}

HC, healthy control.
[a]The LMR HR is presented for each increase of 1/15 unit of LMR and is adjusted for age, sex, and family cluster.