Clinical and Microbiological Effect of a Multispecies Probiotic Supplementation in Celiac Patients With Persistent IBS-type Symptoms

A Randomized, Double-Blind, Placebo-controlled, Multicenter Trial

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Goals: The goals of this study were to evaluate the efficacy and safety of a probiotic mixture in patients with celiac disease (CD) with irritable bowel syndrome (IBS)-type symptoms despite a strict gluten-free diet (GFD).

Background: About 30% of patients with CD adherent to a GFD suffer from IBS-type symptoms; a possible cause resides in the imbalances of the intestinal microbiota in CD. Probiotics may represent a potential treatment.

Study: CD patients with IBS-type symptoms entered a prospective, double-blind, randomized placebo-controlled study. A 6-week treatment period was preceded by a 2-week run-in and followed by a 6-week follow-up phase. Clinical data were monitored throughout the study by validated questionnaires: IBS Severity Scoring System (IBS-SSS); Gastrointestinal Symptom Rating Scale (GSRS); Bristol Stool Form Scale (BSFS); and IBS Quality of Life Questionnaire (IBS-QOL). The fecal microbiota were assayed using plate counts and 16S rRNA gene-based analysis.

Results: In total, 109 patients were randomized to probiotics (n = 54) or placebo (n = 55). IBS-SSS and GSRS decreased significantly in patients receiving probiotics, as compared with placebo ([−15.9% ± 14.8% vs. 8.2% ± 25.9%; P < 0.001] and [−19.8% ± 16.6% vs. 12.9% ± 31.6%; P < 0.001], respectively. Treatment success was significantly higher in patients receiving probiotics, as compared with placebo (15.3% vs. 3.8%; P < 0.04). Presumptive lactic acid bacteria, Staphylococcus and Bifidobacterium, increased in patients receiving probiotic treatment. No adverse events were reported.

Conclusions: A 6-week probiotic treatment is effective in improving the severity of IBS-type symptoms, in CD patients on strict GFD, and is associated with a modification of gut microbiota, characterized by an increase of bifidobacteria.

Key Words: probiotics, celiac disease, gluten-free diet, gut microbiota

Celiac disease (CD) is an autoimmune disorder that occurs in genetically predisposed individuals who develop an immune reaction to gluten, characterized by gluten-dependent clinical manifestations, specific antibodies, HLA-DQ2 and/or DQ8 haplotypes, and enteropathy. Although the adherence to a strict gluten-free diet (GFD) usually leads to the remission of major clinical symptoms, irritable bowel syndrome (IBS)-type symptoms occur frequently in patients with CD despite a strict GFD.

The association of CD and IBS-type symptoms is biologically plausible, with several mechanisms being involved, such as motor dysfunction, low-grade gut inflammation, small intestinal disease, visceral hypersensitivity, and, recently, altered gut microbiota.

Gut microbiota play a key role in maintaining intestinal homeostasis and promoting health. Several recent studies report imbalances in the intestinal microbiota of patients with CD, mainly characterized by an increase of gram-negative bacteria (Bacteroides and enterobacteria) and a decrease of gram-positive bacteria, such as bifidobacteria. It is still debated whether such modifications enter in the pathogenesis of the disease or are just a consequence; however, the intestinal dysbiosis persists irrespective of the adherence to a GFD and in part is related to this particular diet. Indeed, GFD influences gut microbiota composition mainly because of a reduction in polysaccharide intake, which constitutes one of the main energy sources for commensal components of the gut microbiota.
Wacklin et al\(^9\) have shown that dysbiosis of gut microbiota is associated with persistent gastrointestinal (GI) symptoms in treated CD patients, opening new possibilities to treat this subgroup of patients. On the basis of these observations, we hypothesized that probiotics might exert a beneficial effect in the treatment of IBS-type symptoms in patients with CD. The aim of the present study was to evaluate the efficacy and safety of a new probiotic mixture in a randomized, double-blind, placebo-controlled trial in CD patients with IBS-type symptoms despite a strict GFD.

**MATERIALS AND METHODS**

This was a prospective, double-blind, randomized placebo-controlled parallel group study. The patient recruitment was carried out between 2013 and 2015 at the Gastroenterology Units of University of Bari, Castellana Grotte (BA), Foggia and Taranto, by inviting volunteer adult (age 18 y and above) CD patients, who had been treated for a long term (GFD ≥2 y), to participate to the study.

Inclusion criteria for patients were as follows: (a) they complained of persistent IBS-type symptoms according to the ROME III criteria with no clinical evidence of other medical conditions to explain the symptoms,\(^10\) (b) were strictly adherent to a GFD, and (c) had the diagnosis confirmed by chart review, showing elevated serum tissue transglutaminase immunoglobulin-A (tTG-IgA), in the presence of histologic evidence of villous atrophy with crypt hyperplasia, and an increase in intraepithelial lymphocytes on a gluten-containing diet.\(^11\)

Exclusion criteria were as follows: clinically significant cardiovascular, respiratory, endocrine, renal, hematologic, hepatic, neurological or psychiatric disease; previous GI malignancy and/or surgery; pregnancy or lactation; alcohol abuse or drug addiction; current use of medications including corticosteroids or anti-inflammatory drugs, proton-pump inhibitors, antibiotic treatment, and participation in another study. To assess compliance to study treatment and to record adverse events, patients were interviewed on a regular basis by medical personnel blinded to the regimen of each patient. Compliance was calculated as the percentage of ingested study product, and a rate >80% was set as the minimum.

From week 1 to 14, patients filled-in the following questionnaires at 2-week intervals:

1. IBS-SSS consists of 5 questions that generate a maximum score of 100, each using a visual analogue scale and leading to a total possible score of 500. Mild, moderate, and severe cases were indicated by scores of 75 to 175, 175 to 300, and >300, respectively; controls scored below 75, and patients scoring in this range can be considered to be in remission.\(^13\)

2. The 15-item Gastrointestinal Symptom Rating Scale (GSRS), a structured and validated questionnaire widely used in the research of GI diseases, was used to assess the severity of current GI symptoms.\(^14\)

3. Bristol Stool Form Scale (BSFS): stool frequency and type of stool passed were recorded with the BSFS being the reference, which was provided to the parents.\(^15\)

4. Irritable Bowel Syndrome Quality of Life Questionnaire (IBS-QOL): this is a well-established 34-item measure assessing the degree to which IBS interferes with patient quality of life. Each item is rated on a 5-point Likert scale, yielding a total score that ranges from 34 to 170, with higher scores indicating worse QOL.\(^16\)

**Outcome Measures, Adverse Events, and Disallowed Medication**

The primary outcome was to determine whether probiotics, as compared with placebo, were able to improve GI symptoms, as assessed by IBS-SSS; this test was preferred to GSRS, because most of the patients mention IBS-type symptoms, and one third of the score of GSRS refers to the upper GI tract.

Secondary outcomes were to investigate whether probiotics, as compared with placebo, were able to (1) decrease at least 50% the symptom scores, as assessed by IBS-SSS (treatment success); (2) improve GSRS; (3) modify stool parameters, as assessed by BSFS; (4) improve IBS-QOL; and (5) modify gut microbiota and metabolomic fecal profile.

Adverse events were monitored throughout the study. Patients were not allowed to consume any probiotic, other than those provided, or prebiotics, and they were instructed to continue their eating and physical exercise habits. Concomitant use of medications affecting GI motility and/or

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pain perception was allowed, provided that the patients 
registered the intake.

Before and during the trial, staff members instructed 
patients to guarantee a well-balanced diet, with proper gluten 
avoidance, without modification to the dietetic habits.

**Fecal Microbiota**

Fecal samples were collected at the end of week 2, 8, and 
14. Fecal samples (5 g) were mixed with 45 mL sterilized phys-

iological solution and homogenized. Counts of viable bacterial 
cells were carried out as described by De Angelis et al.17

Fecal samples were also used for DNA and RNA 
extractions.17 The sample was subjected to mechanical dis-
ruption with the FastPrep instrument (BIO 101), and total 
DNA was extracted with the FastDNA Spin Kit for Soil (MP 
Biomedicals, Illkirch, France), according to the manu-

facturer’s instructions. An aliquot of ca. 200 mg of fecal 
sample was used for RNA extraction with the stool total 
RNA purification kit (Norgen Biotek Corp., ON, Canada). 
An aliquot of 1 μg of total RNA extracted was transcribed to 
cDNA, using random examers and the tetro cDNA synthesis 
kit from Bioline (Bioline USA Inc., Tanunton, MA), 
according to the manufacturer’s instructions.18

For each subject, the 3 DNA samples, were pooled and 
used for 16S-based tag-encoded FLX amplicon pyro-
sequencing analysis. 16S-based tag-encoded FLX amplicon 
pyrosequencing was performed by Research and Testing 
Laboratories (Lubbock, TX), according to standard labo-

ratory procedures, and using the 454 FLX Sequencer (454 
Life Sciences, Branford, CT).17,19 Raw sequence data were 
screened, trimmed, and filtered with default settings, using 
the QIIME pipeline version 1.4.0 (http://qiime.sourceforge. 
net). Chimeras were excluded by using the B2C2 (www. 
researchandtesting.com/B2C2.html).20 Sequences <250 bp 
were removed. Sequences are available at www. 
researchandtesting.com/docs. FASTA sequences for each 
sample, without chimeras, were evaluated using BLASTn 
against a database derived from GenBank (http://ncbi.nlm. 
nih.gov).21 DNA was also amplified using primer pair 
Probio_ Uni and/Probio_ Rev, which targets the V3 region of 
the 16S rRNA gene sequence.22 DNA was amplified under 
the polymerase chain reaction conditions described 
previously.22 Sequencing analyses were carried out accord-

ing to the protocol of the Ion Torrent PGM system and 
using the Ion Sequencing 200 kit. The sequences were first 
clustered into operational taxonomic unit clusters with 97% 
identity (3% divergence), using USEARCH.23 To determine 
the identities of bacteria, sequences were first queried, using 
a distributed BLASTn.NET algorithm24 against 16S bacte-
rial sequences, which were derived from NCBI. Database 
sequences were characterized as high quality on the basis of 
criteria that were originally described by Ribosomal Data-
base Project (RDP, version 10.28).25 Alpha diversity (rar-

efaction, Good’s coverage, Chao1 richness, and Shannon 
diversity indices) and beta diversity measures were calcu-
lated and plotted using QIIME.

The study adhered to the Declaration of Helsinki and was 
approved by the institutional ethical committee. The 
full trial protocol can be accessed at www.ClinicalTrials.gov 
(identifier NCT01699191). All study participants provided 
written informed consent.

**Statistical Analysis**

All data are expressed as median ± SD with 95% confidence 
intervals unless differently specified. Given the 
exploratory nature of the study, the sample size calculation 
was based on the intent to detect a 25% difference in the 
proportion of responders between treatment and placebo 
groups; 50 patients for each arm were required on the 
basis of a 0.90 power and a 2-sided type 1 error rate of 
5% while compensating for just over a 10% drop out rate.

The χ² test or the Fisher exact test was used, as appropriate, 
to compare percentages and nominal variables. For con-
tinuous variables, differences between patients in the

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**FIGURE 1.** Study design. BSFS indicates Bristol Stool Form Scale; GRSRS, Gastrointestinal Symptom Rating Scale; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; QOL, Quality of Life; R, randomization.
2 treatment arms were compared using analysis of variance, and the Wilcoxon test was used for comparison of the mean values. All statistical tests were 2 tailed and performed at the 5% level of significance. All analyses were performed on the intention-to-treat basis and per protocol analysis. The statistical analyses were performed using the JMP SAS Institute program version 9 (in Cary, NC).

**RESULTS**

The flow of patients involved in the trial from assessment for eligibility through follow-up is shown in Figure 2. Of the 279 patients who participated in the first visit, 161 were excluded, because they did not meet the inclusion/exclusion criteria. Of the remaining 118 patients, 5 (4.2%) refused to participate, and 113 entered the running-in phase without protocol deviations; 3 patients referred a significant improvement of symptoms (IBS-SSS < 75) and exited the study, whereas 1 withdrew consent. Therefore, 109 were available for randomization: 54 were assigned to probiotics and 55 to placebo. At the final assessment, complete data were available for 105 of the 113 participants (93%): 3 patients were noncompliant (2 placebo and 1 probiotics), and 1 was lost to follow-up (probiotics). These 4 patients were excluded from further analysis. The baseline characteristics of the participants in the 2 groups are reported in Table 1. By chance, patients in the probiotic group had significantly worse clinical scores, as compared with those receiving placebo. No differences in the composition of the diet and in the amount of FODMAP consumption were detected during the trial. No adverse events related to the study product were reported.

**IBS-SSS**

The IBS-SSS was at baseline 295 ± 84.9 in the probiotic and 237.6 ± 86.5 in the placebo group ($P < 0.01$); at the end of treatment, it decreased to 170.7 ± 53.4 and 200.8 ± 74.4, respectively ($P < 0.008$). When expressed as variation over the pretreatment value, IBS-SSS in probiotics was significantly decreased, as compared with placebo ($−21.4% ± 15.5%$ vs. $−6.8% ± 21.7%$; $P < 0.001$). At the end of follow-up, IBS-SSS

![FIGURE 2. Flow diagram of patients in the trial from eligibility to the end of follow-up. GFD indicates gluten-free diet; TTG-IgA, tissue transglutaminase immunoglobulin-A.](image-url)

| TABLE 1. Baseline Characteristics of the Patients in the Full Analysis Set |
|---|
| Demographics and Scores | Probiotics (n = 54) | Placebo (n = 55) | $P$ |
| Age (y)* | 43.3 (18.8-62.2) | 44.6 (19.3-63.4) | NS |
| Male/female | 6/35 | 9/46 | NS |
| BMI (kg/m²) | 22.8 ± 3.5 | 23.4 ± 2.9 | NS |
| Positive EMA | 0 | 0 | NS |
| TTG-IgA (IU/mL)* | 0.8 (0-1.2) | 0.5 (0-2.1) | NS |
| Duration of GFD (y)* | 6.8 (2.6-16.7) | 7.4 (3.5-17.5) | NS |
| IBS-SSS | 295 ± 84.9 (95% CI, 269-320) | 237.6 ± 86.5 (95% CI, 211-263) | 0.01 |
| GSRS | 18.7 ± 5.8 (95% CI, 14.6-26.1) | 14.9 ± 5.1 (95% CI, 13.4-27.5) | 0.02 |
| Bristol Stool Charts | 2.6 ± 1.2 | 2 ± 1.5 | NS |
| IBS-QOL | 33.7 ± 17 (95% CI, 28.6-38.9) | 31.5 ± 19.3 (95% CI, 25.7-37.2) | NS |

*Median (range).

TTG-IgA normal value <10 IU/mL.
BMi indicates body mass index; CI, confidence interval; EMA, anti-endomysial antibodies; GFD, gluten-free diet; GSRS, Gastrointestinal Symptom Rating Scale; IBS-QOL, Irritable Bowel Syndrome Quality of Life; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; NS, not significant; TTG-IgA, tissue transglutaminase immunoglobulin-A.
was 176.7 ± 45.1 in the probiotic and 173.6 ± 45.5 in the placebo group, respectively (P = NS).

**GSRS, BSFS, and IBS-QOL**

The GSRS was at baseline 18.7 ± 5.8 in the probiotic and 14.9 ± 5.1 in the placebo group (P < 0.02); at the end of treatment, GSRS changed to 12.2 ± 5.5 and 16.7 ± 6.7, respectively (P < 0.007) (Table 2). When expressed as variation over the pretreatment value, GSRS in probiotics was significantly decreased, as compared with placebo (−19.8 ± 16.6% vs. 12.9% ± 31.6%; P < 0.001). At the end of follow-up, GSRS was 10.1 ± 4.1 in the probiotic and 9.6 ± 4.2 in the placebo group (P = NS).

After treatment, BSFS scores did not differ in patients receiving probiotics or placebo as absolute values (2.2 ± 1.3 vs. 3.1 ± 1.9, respectively; P = NS). When expressed as variation over the pretreatment value, BSFS score in probiotics was significantly decreased, as compared with placebo (−3.3% ± 41.6% vs. 51.5% ± 101.1%; P < 0.010). At the end of follow-up, BSFS scores were 2.3 ± 1.5 in the probiotic and 2.9 ± 1.6 in the placebo group, respectively (P = NS).

After treatment, IBS-QOL scores did not differ in patients receiving probiotics or placebo, either as absolute values (26.7 ± 18.7 and 24.5 ± 16, respectively; P = NS) or when expressed as variation over the pretreatment values (−3.4% ± 37.2% vs. −4.5% ± 19.2%; P = NS). At the end of follow-up, QOL was 23 ± 14.6 in the probiotic and 24 ± 15.7 in the placebo group, respectively (P = NS).

**Treatment Success**

Treatment success was significantly higher in patients receiving probiotics, as compared with placebo, at both intention-to-treat (14.8% vs. 3.6%; P < 0.04) and per protocol (15.3% vs. 3.8%; P < 0.04) analysis; according to our data, 9 patients need to be treated to reach treatment success in 1 (number needed to treat: 9).

**Enumeration of Fecal Cultivable Bacteria and Microbiome**

Compared with baseline value (W2), total anerobes increased from 7.02 to 8.35 log CFU/g (median values, P = 0.018) after 6 weeks of treatment with probiotics. The treatment with probiotics drives also the increase of

| Cultivable Bacteria | W2          | W8          | W14         | W2          | W8          | W14         | W2          | W8          | W14         |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|

**TABLE 3.** Median Values and Range of Cultivable Bacterial Cells (log CFU/g) of the Main Microbial Groups in the Fecal Samples of Celiac Disease With Irritable Bowel Syndrome Patients at Baseline (W2), After 6 Weeks (W8) of Treatment With Probiotics (Pentabiocel) or Placebo, and at the End of Follow-up (W14)

| Cultivable Bacteria | Pentabiocel | Placebo |
|---------------------|-------------|---------|
|                      | W2 vs. W8   | P       | W2 vs. W8   | P       | W2 vs. W8   | P       |

Bold value indicates statistically significant.
presumptive lactic acid bacteria (Lactobacillus, Lactococcus, and Streptococcus), Staphylococcus and Bifidobacterium. Compared with W2, higher level of presumptive Bifidobacterium was also found after 6 weeks (W8) of treatment with probiotics. On the contrary, no statistical difference ($P > 0.05$) was found between the cultivable microbes detected at W2, W8, and W14 for the placebo group (Table 3).

The total bacterial community richness was analyzed by rarefaction curves (Fig. S1, Supplemental Digital Content 1, http://links.lww.com/JCG/A401), richness estimator (Chao1) (Fig. S2A, Supplemental Digital Content 2, http://links.lww.com/JCG/A402), and diversity index (Shannon) (Fig. S2B, Supplemental Digital Content 2, http://links.lww.com/JCG/A402). No statistically significant ($P > 0.05$; false discovery rate $> 0.05$) differences were found between probiotic and placebo groups. A similar trend was also found for metabolically active fecal microbiome (data not shown).

According to alpha diversity, the 3 phylogeny-based beta-diversity measures did not show clear separation between the microbiome composition of placebo and probiotic groups in UniFrac distance principal coordinates analysis plots (Fig. S3, Supplemental Digital Content 3, http://links.lww.com/JCG/A403).

No significant ($P > 0.05$; false discovery rate $> 0.05$) differences were found for the total bacterial phyla (data not shown) and relative abundances of genera between placebo and probiotic groups (Fig. S4, Supplemental Digital Content 4, http://links.lww.com/JCG/A404). Similar results were found for metabolically active bacteria. The only exception was the phylum Actinobacteria and the related genus Bifidobacterium, which was higher in the probiotic compared with the placebo group (Fig. 3). Compared with W2, higher relative amount of metabolically active Bifidobacterium was found in the probiotic group during the washout period (W8) (Fig. 4).
activity of disease. The GFD is able to restore in part the gut microbiota; indeed, while increased numbers of enterobacteria or staphylococci are restored, other alterations such as decreased bifidobacteria and lactobacilli and increased Bacteroides and virulent E. coli are still to persist and, therefore, could play a more prominent role in the maintenance of symptoms.7,8,30,31 The demonstration that a GFD itself determines marked reduction in intake of naturally occurring fructans, which have prebiotic action, might in part explain the reduction in beneficial gut bacteria populations, and may constitute a variable to be considered in treated CD patients, for its possible effects on gut health.33

The presence of a sustained intestinal dysbiosis might be associated with inadequate clinical response and persistence of GI symptoms. To address this hypothesis, Wacklin et al27 analyzed the gut microbiota of CD patients on a strict GFD with persistent symptoms, and found a higher abundance of proteobacteria and a lower abundance of bacte- roidetes and firmicutes, as compared with patients without symptoms, suggesting that intestinal dysbiosis might play a role.

Whether or not these alterations are a cause or a consequence of CD, the similarity of microbiota modification among patients with CD and IBS34 might in part explain the presence of an overlap of clinical manifestation of these 2 conditions.28 Interestingly, in IBS, it has been shown that a state of mucosal immune activation is responsible for increased release of mediators acting on epithelial, neuronal, and muscle cells, leading to intestinal dysfunction and symptom development.28 According to some authors, this immune activation may be the consequence of a perturbation of gut microbiota,59 which might also be operating in CD. On the basis of the dysbiosis hypothesis of GI symptoms in celiac patients, we pursued the idea that a mixture of probiotic strains formulated according to the deficiencies revealed by microbiological data in our celiac population30,31,37 might be of help in this particular setting of patients. Therefore, according to our data on the fecal and duodenal microbiota of CD patients, we selected a pool of 3 bifidobacteria and 2 lactobacilli, to restore the alteration of the microbiota. When using a probiotic mixture, it is fundamental to show activity, as different strains can act as antagonists, both inhibiting probiotic activity of other microorganisms and slowing the microbial growth rate.38

Few human clinical trials have been published to study the effect of a probiotic supplementation in CD with controversial results. Smeu-co et al59 randomized 22 adult patients to receive either Bifidobacterium infantis or placebo before the initiation of a GFD, and, although they found no effect on celiac serology and intestinal permeability, patients on B. infantis experienced a significant improvement in GI symptoms, suggesting a role for probiotics in alleviating symptoms in untreated CD. Olvaress et al60 conducted a double-blind, randomized, placebo-controlled trial in 33 children receiving either Bifidobacterium longum CECT 7347 or placebo while on GFD. After 3 months, the authors were able to show, in the probiotic-treated group, a greater height percentile increase, a reduced serum tumor necrosis factor (TNF)-α concentration, and a decrease of the Bacteroides fragilis group bacteria and fecal secretory IgA, suggesting a beneficial effect in celiac children. Klemenek et al61 investigated the effect of 2 B. breve strains (BR03 and B632) on serum interleukin-10 and TNF-α in 49 children with CD on a GFD and found that the probiotic intervention was able to decrease the production of proinflammatory cytokine. Finally,
Harnette et al\textsuperscript{42} randomized 45 celiac patients, reporting only partial symptom improvement despite a strict GFD, to receive either 5 g of VSL#3 or placebo for 12 weeks, and found no changes in the fecal microbiota counts, blood safety parameters, and clinical improvement in symptoms over the course of the study.

We herein report that the probiotic combination, given daily for 6 consecutive weeks, is superior to placebo in decreasing the severity of IBS-type symptoms in patients with CD despite a GFD. We believe that part of this effect might be secondary to a positive modification of gut microbiota shown by the steady and persistent increase of the bifidobacteria count in fecal samples of CD patients. The evidence that both IBS-SSS and GSRS scores significantly decrease in the probiotic group, despite the fact that the values of both tests at entry were higher in those who received the probiotic, as compared with the placebo, further supports the effect of this probiotic combination. No clinically significant improvement on stool appearance was observed between treatment groups.

We believe that our study has some strength. To the best of our knowledge, this is the first clinical trial investigating the effect of probiotics in celiac patients suffering from IBS-type symptoms despite a strict GFD through both a clinical and microbiological study. Our results provide evidence that a long-term intervention on gut microbiota might determine a healthy clinical outcome in patients with symptoms not responsive to a GFD.

The limitations of the present study are that we have been unable to identify an improvement on QOL despite an improvement of symptoms after probiotic consumption; this might be explained by the complexity of the disease, supporting the hypothesis that patients’ perceived health status is multidimensional, and depends not only on symptoms but also on how diet restriction interacts with other factors, in particular, social life.\textsuperscript{43} Finally, our study is mainly based on subjective evaluations, and we did not perform intestinal biopsies to investigate the link between persistence of symptoms and markers of mucosal inflammation. We do not have data to support the hypothesis that the modification of gut microbiota might persist beyond the period of observation.

In recent years, an increasing amount of data have appeared with regard to the role of gut microbiota in CD patients, suggesting that dysbiosis could result in a modification of the mucosal homeostasis, causing persistent immune activation and clinical symptoms. If we consider the alterations of gut microbiota as an environmental factor involved in CD expression, probiotic administration may have a primary role in the overall manifestation of the disease.

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