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Basic Study

Modulation of faecal metagenome in Crohn's disease: Role of microRNAs as biomarkers

María Rojas-Feria, Teresa Romero-García, Jose Ángel Fernández Caballero-Rico, Helena Pastor Ramírez, Marta Avilés-Recio, Manuel Castro-Fernandez, Natalia Chueca Porcuna, Manuel Romero-Gómez, Federico García, Lourdes Grande, José A Del Campo

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Abstract

BACKGROUND

The gut microbiota plays a key role in the maintenance of intestinal homeostasis and the development and activation of the host immune system. It has been shown that commensal bacterial species can regulate the expression of host genes. 16S rRNA gene sequencing has shown that the microbiota in inflammatory bowel disease (IBD) is abnormal and characterized by reduced diversity. MicroRNAs (miRNAs) have been explored as biomarkers and therapeutic targets, since they are able to regulate specific genes associated with Crohn's disease (CD). In this work, we aim to investigate the composition of gut microbiota of active treatment-naïve adult CD patients, with miRNA profile from gut microbiota.

AIM

To investigate the composition of gut microbiota of active treatment-naïve adult CD patients, with miRNA profile from gut microbiota.

METHODS

Patients attending the outpatient clinics at Valme University Hospital without relevant co-morbidities were matched according to age and gender. Faecal samples of new-onset CD patients, free of treatment, and healthy controls were collected. Faecal samples were homogenized, and DNA was amplified by PCR using primers directed to the 16S bacterial rRNA gene. Pyrosequencing was performed using GS-Junior platform. For sequence analysis, MG-RAST server with the database Ribosomal Project was used. MiRNA profile and their relative abundance were analyzed by quantitative PCR.

RESULTS

Microbial community was characterized using 16S rRNA gene sequencing in 29 samples ($n = 13$ CD patients, and $n = 16$ healthy controls). The mean Shannon diversity was higher in the healthy control population compared to CD group (5.5 *vs* 3.7). A reduction in *Firmicutes* and an increase in *Bacteroidetes* were found. *Clostridia* class was also significantly reduced in CD. Principal components analysis showed a grouping pattern, identified in most of the subjects in both groups, showing a marked difference between control and CD groups. A functional metabolic study showed that a lower metabolism of carbohydrates ($P = 0.000$) was found in CD group, while the metabolism of lipids was increased. In CD patients, three miRNAs were induced in affected mucosa: mir-144 (6.2 ± 1.3 fold), mir-519 (21.8 ± 3.1) and mir-211 (2.3 ± 0.4).

CONCLUSION

Changes in microbial function in active non-treated CD subjects and three miRNAs in affected *vs* non-affected mucosa have been found. miRNAs profile may serve as a biomarker.

Key words: Crohn's disease; Dysbiosis; microRNAs; *Firmicutes*; *Bacteroidetes*

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Core tip: In this study, we have found a shift in microbial gut community composition that supports dysbiosis in Crohn's disease (CD) patients. The greatest interest of our work is that we have only included new-onset adult CD patients. We found that active non-treated CD patients had a low *Firmicutes*/*Bacteroidetes* ratio, less biodiversity in the structure of microbial communities and a significantly different pattern on gut microbiota distribution. Three microRNAs (miRNAs) have been found induced in affected mucosa *vs* non-affected mucosa in CD, indicating that miRNA profile may serve as biomarker for active disease.

Rojas-Feria M, Romero-García T, Fernández Caballero-Rico JÁ, Pastor Ramírez H, Avilés-Recio M, Castro-Fernandez M, Chueca Porcuna N, Romero-Gómez M, García F, Grande L, Del Campo JA. Modulation of faecal metagenome in Crohn's disease: Role of microRNAs as biomarkers. *World J Gastroenterol* 2018; 24(46): 5223-5233

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INTRODUCTION

Up to now, the pathogenesis of inflammatory bowel disease (IBD) has not been clarified. A plausible theory is that IBD develops in genetically susceptible individuals due to the abnormal immune response against luminal antigens and microbiota^[1]. The targets of this response are thought to be antigens derived from constituents of the microbiota.

The gut microbiota plays a key role in the maintenance of intestinal homeostasis and the development and activation of the host immune system. The composition of microbiota community evolves during the first years of life, increasing the microbial diversity gradually. During this evolution, the host genetics and the environmental factors can shape the microbiome composition.

On the other hand, it has been shown that commensal bacterial species can regulate the expression of host genes. 16S rRNA gene sequencing has shown that the microbiota in IBD is abnormal and characterized by reduced diversity. The causality between IBD and alterations in microbiota remains incompletely understood but one theory is that altered microbiota composition and function in IBD result in increased immune stimulation or enhanced mucosal permeability.

A strong genetic component has been described in IBD, with the identification of about 200 loci associated with the development of the disease^[2]. However, this can only explain a 16%-23% of the heritable of IBD^[3-5]. Epigenetic factors can mediate interactions between the environment and the genome and could therefore play a central role in the pathogenesis of IBD and other

diseases^[6]. Epigenetic can be defined as heritable changes in gene function not explained by changes in DNA sequence. One of the main epigenetic mechanisms includes RNA interference, transmitted by microRNAs (miRNAs). MiRNAs are noncoding single stranded RNAs that regulate gene expression at the posttranscriptional level, influencing numerous biological processes, as cell proliferation, differentiation and death. A large number of miRNAs (> 1600) has been described in humans. Each miRNA can regulate multiple genes and a single gene may be targeted by many different miRNA^[6]. A challenge is to identify which of the multiple miRNA that regulate a gene has an essential role.

In IBD, research focusing on miRNA did not begin until 2008. Wu *et al*^[7] carried out the first study in which they identified miRNA profile in intestinal biopsies from IBD patients. Since then, and over the last decade, studies related to miRNA have increased exponentially, focusing mainly on three aspects: the alteration of miRNAs in the different IBD profiles, their therapeutic role and their pathway. Likewise, these studies have tried to identify miRNA in different tissues: colon, blood, and saliva^[8], as a goal to find an effective non-invasive test for disease. MiRNAs have been involved in the pathogenesis of IBD and have been explored as biomarkers and therapeutic targets^[9]. It has been shown that miRNAs regulate specific genes associated with Crohn's disease (CD) including nucleotide-binding oligomerization domain-containing protein 2 (NOD2), interleukin (IL)-6 and tumor necrosis factor (TNF).

Cao *et al*^[9] described the role of miRNA on intestinal epithelial barrier deregulation, and disruption of immune homeostasis, leading the development and progression of the disease. Apart from that, miRNAs have been proposed as biomarkers of IBD, useful in differential diagnosis of IBD, and as a tool of treatment evaluation and prognosis^[10].

Identification of host and microbiota alterations in individual patients should lead to selective target interventions. In this study, we first analyzed the faecal microbiota composition in CD patients at the time of diagnosis. Secondly, we compared miRNA expression in CD gut samples obtained from endoscopically normal and affected mucosa, in order to find a marker of active IBD.

MATERIALS AND METHODS

Patients

Consecutive new-onset adult CD patients, free of treatment, that underwent colonoscopy for diagnostic purposes, were included in this study. Informed consent was obtained from all of them. Faecal samples from 13 patients (9 females, 67%; mean age 32.18 + 14.8 years old) and 16 healthy controls, matched by age and gender, were also collected. Inflammatory behaviour was present in 77% (10/13) of patients, stenotic in 23% (3/10) and fistulizing in 7.6% (1/13). None of the patients or the controls received antibiotic treatment the

10 wk before of recruitment.

A total of 35 gut biopsy samples from the subset of patients with non-fistulizing non-stenotic phenotype were taken (17 from affected mucosa and 18 from healed mucosa), immediately frozen in liquid nitrogen and submitted for miRNA study. Four patients had ileal, 3 colonic and 3 ileocolonic diseases.

DNA extraction, quantification and pyrosequencing analysis

Faecal samples were homogenized in a Stomacher-400 mixer. The DNA was extracted from stool using the QIAamp DNA Mini Kit (QIAGEN, Barcelona, Spain) as indicated by the manufacturer, with the exception that the samples were mixed with the lysis buffer and incubated at a temperature of 95 °C instead of 70 °C to ensure the lysis of Gram-positive and negative bacteria. DNA quantification was carried out by the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, DE, United States). The extracted DNA was amplified by PCR using primers targeting the targets flanking the variable regions of 1 to 3 of the bacterial gene 16S rRNA (V1-3), subsequently gel-purified, and analyzed using the technology of Roche 454 GS FLX (Branford, CT, United States), being the final amplicon obtained is a 600 pb sequence belonging to the variable region V1-V3 of the 16S rRNA gene. PCR was performed in a total volume of 15 µL for each sample, which contains the forward universal primers 27F and Bif16S-F (10 µmol/L) in a ratio of 9:1 respectively, and the universal barcode 534R as primer inverse (10 µmol/L), in addition to the mixture of dNTP (10 mmol/L), FastStart 10 × buffer with 18 mmol/L of MgCl₂, FastStartHiFi polymerase (5 U/mL), and 2 µL of genomic DNA. PCR were performed in a high fidelity thermocycler (Roche AppliedScience, Penzberg, Germany). PCR conditions were the following: 95 °C for 2 min, 30 cycles of 95 °C for 20 s, 56 °C for 30 s, and 72 °C for 5 min and a final stage at 4 °C. PCR products were purified using magnetic beads AMPure XP (Beckman-Coulter, Brea, CA, United States).

DNA quality and concentration were measured using the Quant-IT™ PicoGreen® dsDNA Assay Kit (ThermoFisher, Waltham, MA, United States). Finally, the amplicons are combined in equimolar ratios to create a pool of DNA (109 DNA molecules) that was used for clonal amplification (emPCR) and pyrosequencing according to the manufacturer's instructions. At the end of the massive sequencing process, all the sequences were annotated according to quality, and the sequences with poor quality and short fragments were eliminated.

Sequences were selected to estimate the total diversity of bacteria in the DNA of the samples. For this purpose, bar codes, primers, chimeras, plasmids, mitochondrial DNA, any sequence not belonging to 16S RNA and sequences < 150 bp were eliminated. For the analysis of all the sequences the MG-RAST server (metagenomic analysis server) was used with the database Ribosomal Project (RDP), which converted the total se-

quences into relative abundances of microorganisms of the different taxonomic levels. Processing in MG-RAST includes demultiplexing, quality filtering, length filtering, dereplication and deletion of model organism sequences.

miRNA screening

Total RNA was extracted using the mirVana™ miRNA Isolation Kit, with phenol (ThermoFisher Scientific, MA, United States). The commercially available Kit RT2 miRNA PCR Array System (SABiosciences-Qiagen, Hilde, Germany) was used for the relative quantification of the 88 most expressed miRNAs following the manufacturer's instructions. Individual miRNA gene expression was measured by retrotranscription with the miScript II RT Kit (Qiagen, Hilde, Germany), and a quantitative PCR by the miScript SYBR® Green PCR Kit (Qiagen, Hilde, Germany) by a Stratagene 3005 MxP thermocycler.

Statistical analysis

The MG-RAST output file was analyzed with the SPSS Statistics 20.0 software (SPSS Inc), while the STAMP software version 2.1.3 was used for the metagenomic analysis and IMG/M. The Shannon index, based on species richness (number of species present) and species abundance (number of individuals per species), was calculated for control samples and samples with CD. The differences between the two groups were compared using the Mann-Whitney *U* test. Statistical analysis was performed using the GraphPadPrism 7 and SPSS 20.0 software. Differences between means were performed with significance tests using an analysis of variance (ANOVA) and post-hoc test with less significance. Non-parametric data were expressed as median (range) and analyzed using the Mann-Whitney *U* test. Differences between proportions were analyzed by chi-square test. Significance was accepted at $P < 0.05$.

RESULTS

Ecological analysis

Microbial community was characterized using 16S *rRNA* gene sequencing in 29 samples ($n = 13$ CD participants and $n = 16$ healthy controls). The mean Shannon diversity was found higher in the healthy control population compared to the CD group (5.5 vs 3.7), indicating that there is less biodiversity in the structure of microbial communities in CD. To check this, we decided to measure the number of species in both groups (357 control group vs 289 CD group), which showed a decreased number of species in the CD group.

Dysbiosis was observed in CD due to an increased population of *Bacteroidetes* and reductions in *Firmicutes*. *Firmicutes/Bacteroidetes* ratio was 1.71 in controls versus 0.80 in CD. Furthermore, the number of readings obtained using 16S massive sequencing is shown in Figure 1A. 77143 readings were obtained in control samples and 69296 reads in CD group.

Metagenomic analysis

To assess how the intestinal microbial community was

distributed in both groups, principal components analysis (PcoA) was performed (Figure 1B). A grouping pattern was identified for most of the subjects in both groups, showing a marked difference between control samples (blue) and CD samples (green). A PERMANOVA calculated by FIRST showed statistically significant differences between groups ($P < 0.05$).

Analysis in bacterial taxonomic groups

Differential distributions in taxonomic categories were analyzed. Significant differences in Clostridia class were found in the analysis, being decreased in the CD group (Figure 2). Significant differences were found in *Entomoplasmataceae* ($P = 0.001$), *Bacteriaceae* ($P = 0.002$), *Lachnospiraceae* ($P < 0.0001$), *Ruminococcaceae* ($P < 0.0001$) and *Rikenellaceae* ($P = 0.003$) (Figure 3).

Relative abundance of the most abundant bacterial genera

Significant differences in *Ruminococcus* ($P < 0.001$), *Roseburia* ($P = 0.002$), *Parabacteroides* ($P = 0.02$), *Mesoplasma* ($P = 0.003$), *Faecalibacterium* ($P < 0.001$), *Eubacterium* ($P = 0.003$) and *Alistipes* ($P < 0.003$) were observed, showing a decreased distribution in the EC group (Figure 4).

When relative abundance of each bacterial taxon was analyzed, all of them were increased in the control group EC except *Clostridium bolteae*, only present in CD while absent in the control group: *Ruminococcus albus* ($P = 0.004$), *Roseburia inulinivorans* ($P = 0.016$), *Mesoplasma lactucae* ($P = 0.002$), *Faecalibacterium prausnitzii* ($P < 0.0001$), *Eubacterium ramulus* ($P = 0.006$), *Eubacterium eligens* ($P = 0.03$), *Eubacterium coprostanoligenes* ($P = 0.002$), *Dialister invisus* ($P = 0.005$), *Desulfonauticus autotrophicus* ($P = 0.01$), *Clostridium* culture spenrichment clone 7-25 ($P < 0.0001$), *Clostridium bolteae* ($P = 0.04$), *Clostridia lesgenomosp.* BVAB3 ($P = 0.02$), *Butyrivibrio fibrisolvans* ($P = 0.04$), butyrate producing bacterium ART55 / 1 ($P = 0.006$), *Bacteroides dorei* ($P = 0.04$), *Alistipes putredinis* ($P = 0.03$), *Alistipes finegoldii* ($P = 0.02$).

Functional analysis

A functional metabolic study of the microbiota was performed by comparison between both groups. We observed significant differences in the following functions: Biosynthesis and glycan metabolism, carbohydrate metabolism, lipid metabolism, catabolism, digestive system, amino acid metabolism, immune system. In the CD group, we observed a lower metabolism of carbohydrates ($P = 0.000$) while the metabolism of lipids was increased in this group ($P = 0.000$). Data is shown on Figure 5.

miRNAs

In order to analyze the miRNA profile in patients with CD, we performed an array of the 88 most abundant miRNAs in human inflammatory processes. After RNA extraction from biopsies, samples were pooled (control vs affected mucosa). In this analysis, 4 miRNAs were found to be significantly induced (fold change > 1.5) in affected

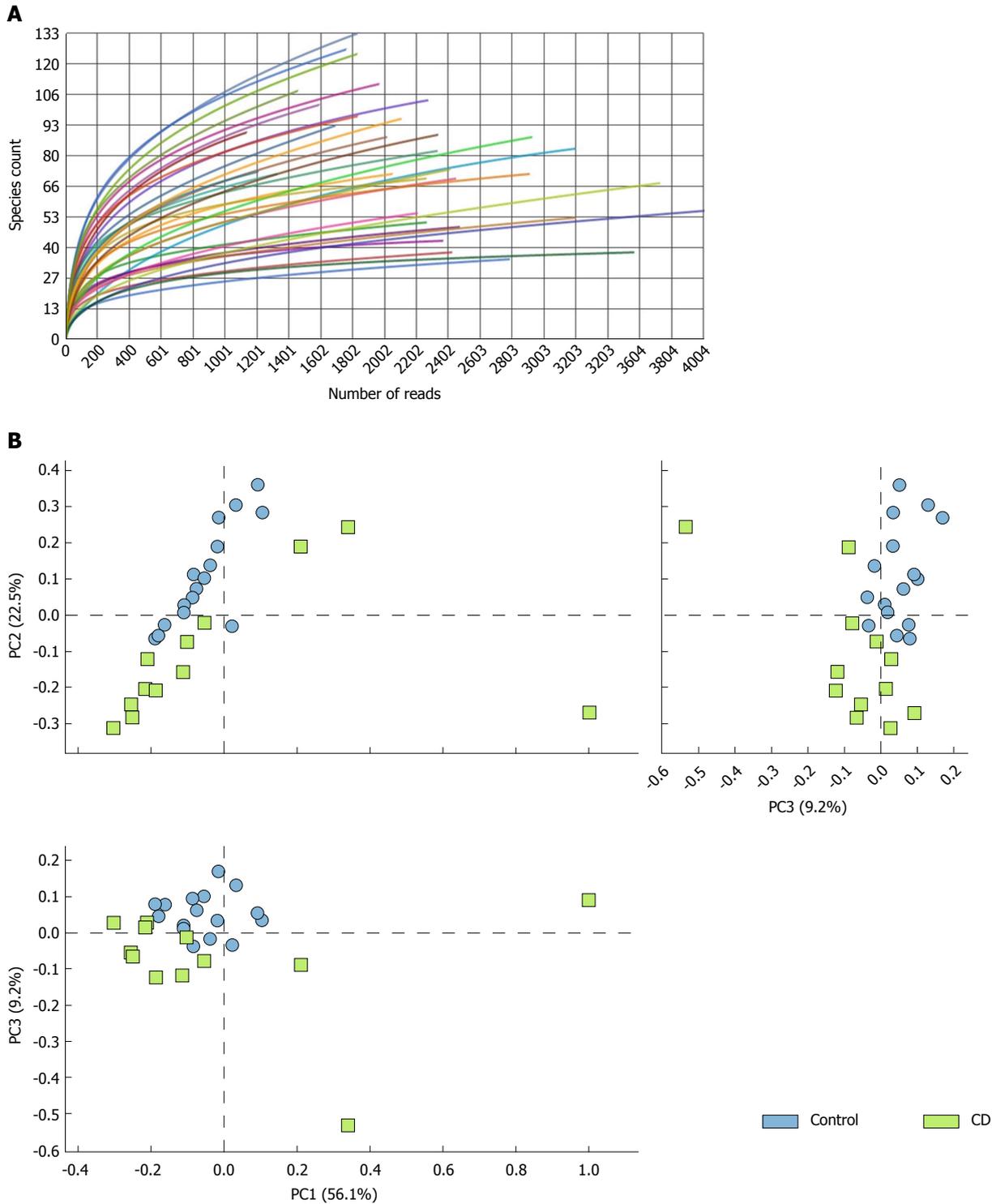


Figure 1 Ecological and metagenomic analysis. A: Number of readings for each sample using the 16S massive sequencing technique in GS Junior. See methods section for details. B: Principal components analysis. The control samples and Crohn's disease are observed in well-defined groups. The data was selected with the Ribosomal Project database using a maximum e-value of 10^{-5} , a minimum identity of 75%, and a minimum length alignment of 15 bp. PC: Principal components; CD: Crohn's disease sample.

mucosa vs non-affected: miR-144, miR-211, miR-373-3p and miR-519.

An individual analysis was performed on 10 additional samples from patients with CD, as a validation analysis, where three of them were also found induced (miR-144, miR-211 and miR-519). Results are shown on Figure 6.

DISCUSSION

In this study, we have found a shift in microbial gut community composition that supports dysbiosis in CD patients. The greatest interest of our work is that we have only included new-onset adult CD patients.

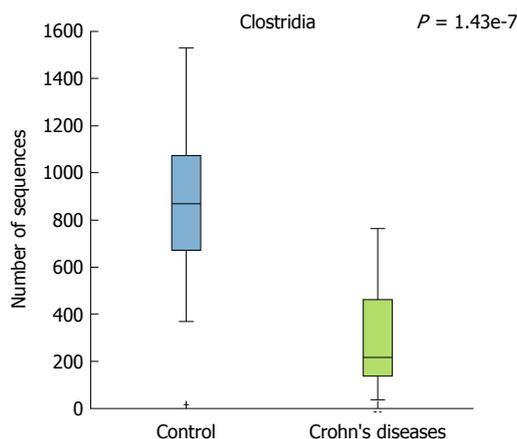


Figure 2 Box plot showing a significant difference in the *Clostridia* class between control group (blue) and Crohn' disease (green).

No confounders of drug therapy, surgery or duration of the disease were present. We described a low proportion of *Firmicutes* population, and a reduction in *Firmicutes/Bacteroidetes* ratio in CD, respect to control samples. In healthy adults, 80% of the identified faecal microbiota belongs to 3 dominant phyla: *Bacteroidetes*, *Firmicutes* (principally *clostridia*) and *Actinobacteria*^[11,12]. *Firmicutes* are considered beneficial and *Bacteroidetes* are considered aggressive, being implicated in the pathogenesis of IBD^[13]. The *Firmicutes* to *Bacteroidetes* ratio has shown to be of significant relevance in gut microbiota composition, as an expression of the degree of dysbiosis^[14]. In healthy adults, ratios of 10.9 were measured^[15], although ratios between 1 and 5.5 were considered optimum. In our population, the ratio was 1.71 in controls vs 0.8 in CD.

Our results are in concordance with data from other authors^[16-18], although some of them analysed mucosa microbiota instead of faecal microbiota. Interestingly, in a former study^[19], the reduction in the *Firmicutes/Bacteroidetes* ratio was more likely to be observed in ulcerated mucosa than in non-ulcerated mucosa. Based on this, it is questionable whether the dysbiosis is not an effect instead of a cause. However, these findings have not been confirmed in later studies^[16].

We have also found less biodiversity and a significantly different pattern on microbiota distribution in active CD patients compared to controls. *Ruminococcus*, *Roseburia*, *Parabacteroides*, *Mesoplasma*, *Faecalibacterium*, *Eubacterium* and *Alistipes* were significantly decreased in our CD cohort. Certain bacteria, including *Bacteroidetes*, *Bifidobacterium*, *Clostridium* clusters XIVa and IVa, *Eubacterium rectale*, *Faecalibacterium prausnitzii*, lactic acid bacteria, *Roseburia intestinalis*, and others, are closely related to gastrointestinal tract fitness^[20]. Therefore, a decrease of these genera could favour the development of CD.

We have described significant differences in *Clostridia* class in the analysis, being decreased in the CD group. This finding is in agreement with other researchers that have identified 43 species of *Firmicutes* in healthy human

gut microbial communities, while only 13 species were found in patients with CD. In addition, the proportion of *Clostridium prausnitzii* in CD patients was significantly lower compared to controls^[21,22]. *Clostridium* species have been shown to induce the expansion of T_{reg} cells, reducing intestinal inflammation. *Faecalibacterium* genera may protect the host from mucosal inflammation through several mechanisms, including down-regulation of inflammatory cytokines^[23] or IL-10 stimulation^[24], an anti-inflammatory cytokine. *Faecalibacterium prausnitzii*, a proposed member of the microbiota with anti-inflammatory properties, is under-represented in IBD, as we have reported in this study.

Clostridium boltae is only present in the CD group. Therefore, this organism could be a target in CD patients as a risk factor for disease development. Despite promising correlations between shifts in microbial composition and CD, the presence or absence of a single taxon has not been identified as causal or protective against the development of disease^[25]. This supports the view that compositional change (dysbiosis), rather than a single putative organism, may be responsible for this disease.

Mucosal-associated microbes are uniquely positioned to influence the immune system; particularly, the porous mucus layer in the ileum has been shown to educate the immune system to develop tolerance towards commensals^[26]. In fact, faecal microbial transplant (FMT) has been proposed as a potential therapeutic option for individuals with CD based on the hypothesis that changes in dysbiosis could lessen intestinal inflammation^[27].

Besides the shifts in microbiota composition, we have identified significant changes in carbohydrate and lipid metabolism, the first being reduced and the second being heightened. Changes in microbial function between healthy controls and IBD appear more extensive and more consistent compared with changes in community structure^[28]. *Clostridium* cluster XIV (*Eubacterium rectale*) and *Clostridium* cluster IV (*Clostridium leptum* group and *Faecalibacterium prausnitzii*) have a function in carbohydrate fermentation and short chain fatty acid (SCFA) production, particularly butyrate^[18]. SCFA are the major source of energy for the gut epithelial cells and possess an anti-inflammatory activity. Butyrate has been found to decrease TNF production and proinflammatory cytokine expression in intestinal tissue in patients with CD^[29]. Taking all this into account, dysbiosis leads to changes in microbiota metabolism, resulting in a permanent inflammation process for gut cells.

To find a biomarker of active CD, miRNAs expression in gut mucosa was analyzed. We have identified differential expression of 3 miRNAs in affected colonic mucosa from patients with naive CD. These miRNAs (miR144-3p; miR-211-5p; miR-519d-3p) are induced in the diseased colonic mucosa compared to the healthy areas. The miRNAs identified in our study have not been previously described elsewhere, although multiple different miRNAs expression profile have already been identified in inflamed and non-inflamed mucosa in

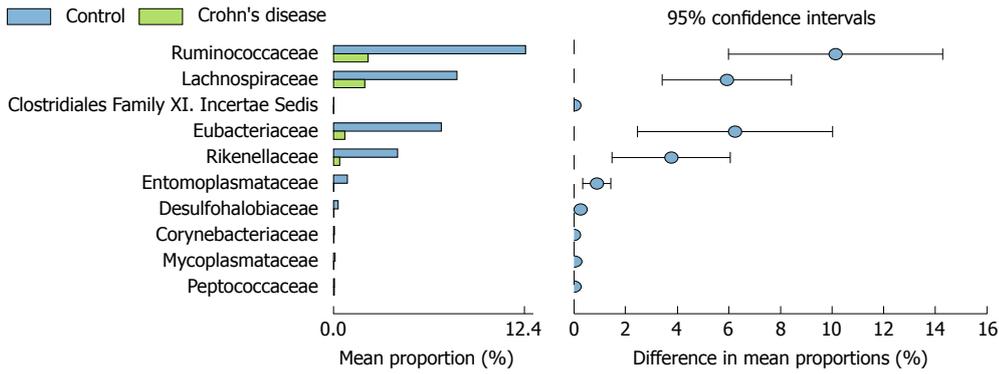


Figure 3 Bar graph with the mean of each group (Crohn's disease and control population) and family taxon (95% confidence level).

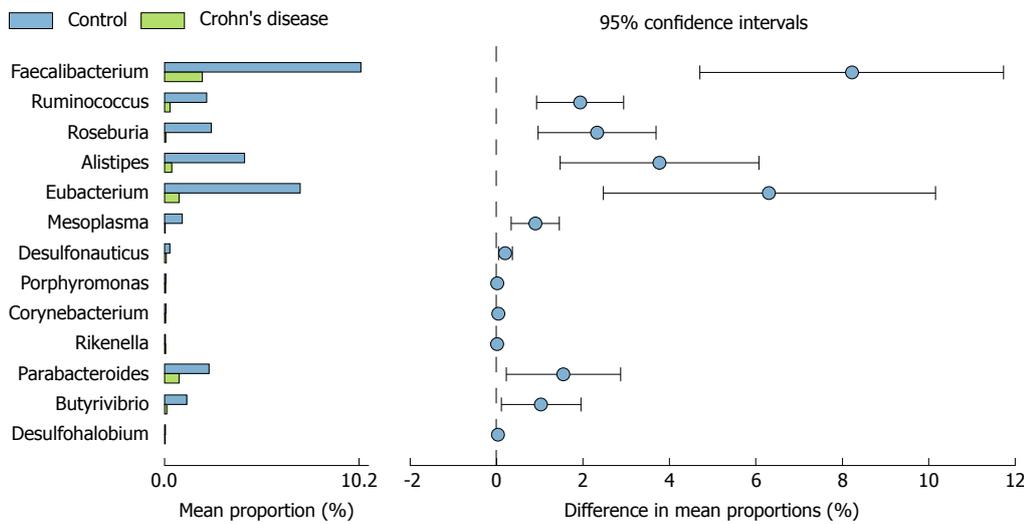


Figure 4 Bar chart with the mean of each taxonomic group (gender) according to group (control vs Crohn's disease) and differences with 95% confidence level.

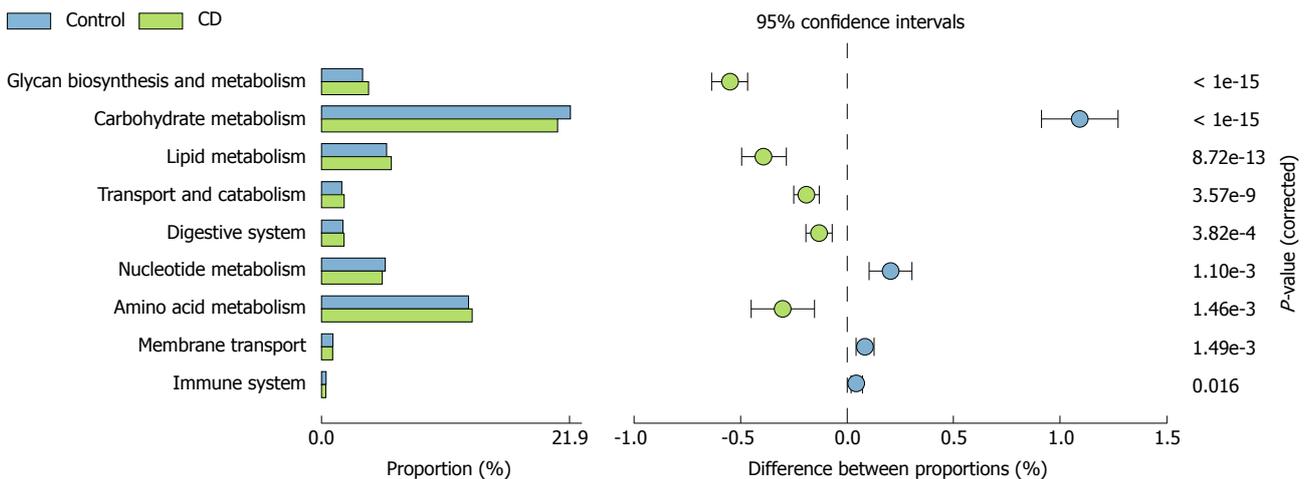


Figure 5 Functional analysis of the microbiota. Significant differences ($P < 0.05$) in biosynthesis and glycan metabolism, carbohydrate metabolism, lipid metabolism, catabolism, digestive system, amino acid metabolism and immune system were found. C: Control sample; CD: Crohn's disease sample.

IBD patients. Some studies have demonstrated up-regulation of miRNAs in inflamed mucosa^[30] whereas others pointed out down-regulation of miRNA in affected mucosa^[31,32]. It has been shown that miR-215 expression in biopsies of CD patients correlates with the

likelihood of disease progression^[10]. Nevertheless, the miRNA expression profile in colonic tissue has not been defined yet. Recently, 100 miRNAs have been identified as significantly deregulated between the inflamed and normal mucosa of ascending colon^[33]. Differentially

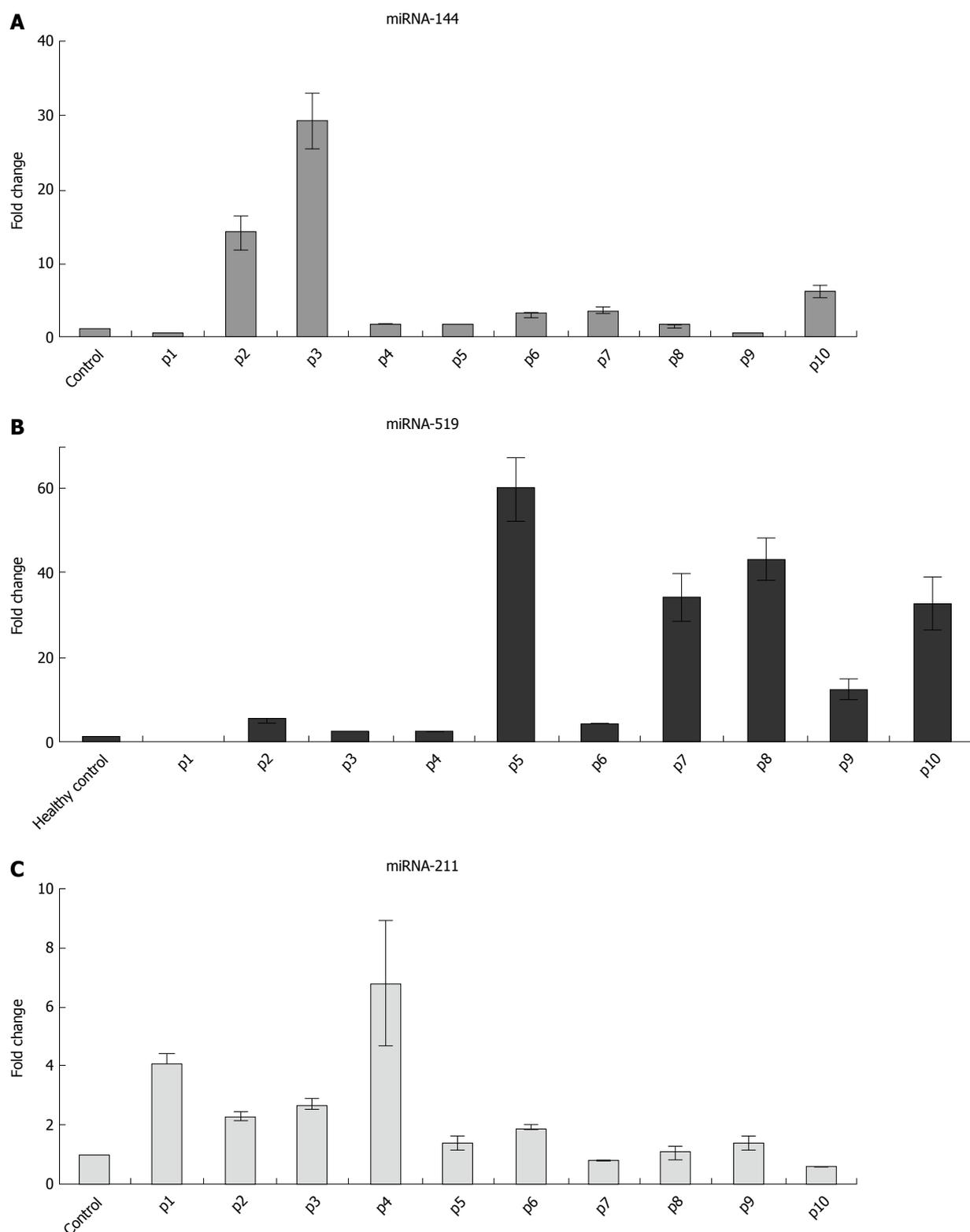


Figure 6 Three microRNAs were found increased in samples from patients with Crohn's disease. Individual microRNA levels in 10 patients with Crohn's disease are represented. miRNA: MicroRNA.

expressed miRNAs identified from these studies and our results may be due to differing factors such as sample size, CD patient characteristics (age, drug history, with or without surgery, *etc.*) and subject population demographics.

It should be noted that although in all our patients

we found significant over-expression of these miRNAs, depending on the patient studied we found one of these increased with respect to the others. Therefore, we hypothesized that the combined analysis of specific miRNAs (in our case, miR144-3p; miR-211-5p; miR-519d-3p) could be useful to determine disease proba-

bility indexes in patients. This idea of miRNA combination analysis has already been proposed in other recent studies^[9]. However, the development of a model including miRNAs combination is still pending.

Although our patient cohort is small, we have carefully selected these patients at the time of diagnosis and all of them had inflammatory phenotype and were naive for treatments. To validate our findings, we will analyze circulating miRNAs, as potential non-invasive biomarkers for diagnosis and disease progression. Some studies have already pointed out this role after infliximab therapy^[34] or exclusive enteral nutrition^[35].

In conclusion, we found that active non-treated CD patients had a low *Firmicutes/Bacteroidetes* ratio, less biodiversity in the structure of microbial communities and a significantly different pattern on gut microbiota distribution. Moreover, microbiota metabolism was altered in CD compared to healthy subjects. This data strongly suggests that dysbiosis may play a role in the pathogenesis of CD. Three miRNAs have been found induced in affected mucosa vs non-affected mucosa in CD, indicating that the miRNA profile may serve as a biomarker for active disease. Nevertheless, additional studies are needed to identify causative roles for the microbiota and to establish the role of miRNAs in the pathogenesis or diagnosis of IBD.

ARTICLE HIGHLIGHTS

Research background

Pathogenesis of inflammatory bowel disease (IBD) has not been clarified yet. The gut microbiota plays a key role in the maintenance of intestinal homeostasis and the development and activation of the host immune system. The causality between IBD and alterations in microbiota remains incompletely understood but one theory is that altered microbiota composition and function in IBD result in increased immune stimulation or enhanced mucosal permeability. On the other hand, microRNAs (miRNAs) have been involved in the pathogenesis of IBD and have been explored as biomarkers and therapeutic targets. It has been shown that miRNAs regulate specific genes associated with Crohn's disease (CD).

Research motivation and objectives

Identification of host and microbiota alterations in individual patients should lead to selective target interventions. In this study, we first analyzed the faecal microbiota composition in CD patients at the time of diagnosis. Secondly, we compared miRNA expression in CD gut samples obtained from endoscopically normal and affected mucosa, in order to find a marker of active IBD.

Research methods

In this study, we will use deep-sequencing methods to analyze the microbiota from patients with CD and healthy controls. Moreover, a miRNAs screening will be performed to identify individual miRNA involved in inflammation process that could serve as biomarkers for disease progression on therapeutic target.

Research results

We found significant differences in microbiota composition when comparing patients with CD compared to the control population. The major differences were found in microbial biodiversity (Shannon Index). We also found a reduction in *Firmicutes* and an increase in *Bacteroidetes*. *Clostridia* class was also significantly reduced in Crohn's disease group.

Research conclusions

We found that active non-treated CD patients had a low *Firmicutes/Bacteroidetes*

ratio, less biodiversity in the structure of microbial communities and a significantly different pattern on gut microbiota distribution. Moreover, microbiota metabolism was altered in CD patients compared to healthy subjects. This data strongly suggests that dysbiosis may play a role in the pathogenesis of CD. Three miRNAs have been found induced in affected mucosa vs non-affected mucosa in CD, indicating that miRNA profile may serve as a biomarker for active disease.

Research perspectives

Additional studies are needed to validate the results obtained and to identify causative roles for the microbiota. The role of miRNAs in the pathogenesis or diagnosis of IBD, including CD must be established through deeper analysis and validation in circulating tissues.

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