Clinical Nutrition 38 (2019) 2304–2310

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Altered host-gut microbes symbiosis in severely malnourished anorexia nervosa (AN) patients undergoing enteral nutrition: An explicative factor of functional intestinal disorders?

Mouna Hanachi [a, b, **], Chaysavanh Manichanh [c, *], Andreu Schoenenberger [c], Victoria Pascal [c], Florence Levenez [d], Nicole Cournède [a], Joël Doré [d, e], Jean-Claude Melchior [a, b]

[a] Clinical Nutrition Unit, Raymond Poincaré University Hospital (AP-HP), Garches, France
[b] Université de Versailles, Saint-Quentin-en- Yvelines, Montigny-le-Bretonneux, France
[c] Department of Gastroenterology, Vall d'Hebron Research Institute, Barcelona, Spain
[d] MetaGenoPolis, INRA, Université Paris-Saclay, Jouy-en-Josas, France
[e] MICALIS Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France

ARTICLE INFO

Article history:
Received 24 January 2018
Accepted 1 October 2018

Keywords:
Undernutrition
Gut dysbiosis
Anorexia nervosa
Functional intestinal disorders

SUMMARY

Background: Functional intestinal disorders (FIDs) are frequently observed in patients with anorexia nervosa (AN). Relationship between FIDs and a potential gut microbiota dysbiosis has been poorly explored.
Objective: We aimed to determine an association between FIDs severity and dysbiosis of the intestinal microbiota in a severely malnourished patient population with AN undergoing enteral nutrition.
Design: Faecal microbiota of AN (DSM IVr criteria) female inpatients were collected and compared to healthy controls based on 16S rRNA profiling. The severity of FIDs was evaluated in patients and healthy controls using Francis Score.
Results: Thirty-three patients (BMI: 11,7 ± 1,5; Age: 32 ± 12) and 22 healthy controls (BMI: 21 ± 2; age: 36 ± 12) were included. A marked dysbiosis was identified in AN patients compared to healthy controls ($p = 0.03$). Some potentially pathogenic bacterial genera (*Klebsiella*, *Salmonella*) were more abundant in AN patients whereas, other bacterial symbionts (*Eubacterium* and *Roseburia*) involved in immune balance were significantly less abundant in patients than controls. Severity of FIDs was strongly correlated with several microbial genera ($r = -0.581$ for an unknown genus belonging to Peptostreptococcaceae family; $r = 0.392$ for *Dialister*, $r = 0.444$ for *Robinsoniella* and $r = 0.488$ for *Enterococcus*). Other associations between dysbiosis, clinical and biological characteristics were identified including severity of undernutrition (BMI).
Conclusion: Observed gut microbiota dysbiosis in malnourished patients with anorexia nervosa is correlated with the severity of FIDs and other metabolic disturbances, which strongly suggests an altered host-microbe symbiosis.

1. Introduction

Anorexia nervosa is a chronic psychiatric disease with potentially serious somatic consequences [1] with a prevalence in general population that varies between 0.9 − 3% and 0.16–0.3% in females and males, respectively [2]. AN is characterized by an emotional and cognitive incapacity to maintain a normal weight and by an active struggle against the sensation of hunger [3]. This behavioral symptomatology leads to important weight loss, undernutrition and more or less severe - potentially life-threatening - somatic complications [1]. Functional intestinal disorders (FIDs), including

Abbreviations: AN, anorexia nervosa; FID, Functional intestinal disorders; BMI, body mass index; IBS, Irritable Bowel Syndrome; 16S rRNA, 16S ribosomal RNA; OTU, operational taxonomic unit; FDR, false discovery rate.

* Corresponding author. Vall d'Hebron Research Institute, Pg Vall d'Hebron 119-129, Barcelona, Spain.
** Corresponding author. Hôpital Raymond-Poincaré, 104 boulevard Raymond Poincaré, 92380, Garches, France.
E-mail addresses: mouna.hanachi@aphp.fr (M. Hanachi), cmanicha@gmail.com (C. Manichanh).

https://doi.org/10.1016/j.clnu.2018.10.004
0261-5614/

constipation, abdominal pain and bloating, are common complications reported by patients with eating disorders; these symptoms are causing psychological stress and delayed renutrition [4]. The prevalence of these intestinal disorders and their intensity seem to worsen along with the duration of the disease and the degradation of nutritional status [5]. Physiopathologic hypotheses including gut microbiota pathway to explain FIDs in malnourished AN patients has not been explored so far. The FIDs described in the Irritable Bowel Syndrome (IBS) are comparable to those described in AN and 41% of patients with AN meet the diagnosis criteria of IBS [6] where a dysbiosis of the intestinal microbiota has been reported [7] and associated with FIDs [8]. Dysbiosis refers to an alteration of the microbial community compared to a healthy state.

We hypothesized that gut microbial community composition and structure alterations may be a determinant of either FIDs intensity and undernutrition severity and other clinical and metabolic abnormalities during severe malnutrition in anorexia nervosa patients.

The study was carried out to investigate the presence of intestinal microbiota dysbiosis in malnourished patients with anorexia nervosa compared to healthy controls; and additionally to explore dysbiosis association with functional intestinal disorders.

2. Material and methods

2.1. Ethics and study registration

All experiments were performed in accordance with relevant guidelines and regulations. Patient and related controls data records were declared to the French data protection authority CNIL (CNIL: 1990894 v 0) and registered at the French Clinical Trial registry (CT: NCT02920073). All participants were informed about the study purpose during the admission interview and provided a written consent prior to inclusion.

2.2. Study design

We conducted an observational, mono-centric, comparative case–control study between January 2013 and June 2015 in the Clinical Nutrition Unit of Raymond Poincaré university hospital (APHP, UVSQ), Garches, France.

2.3. Subjects

2.3.1. Patients

We enrolled adult patients with a diagnosis of AN according to DSM-IVr criteria (patients underwent a structured interview, and their current AN phenotypes were diagnosed), with severe malnutrition defined by a body mass index (BMI: height/weight ^{2}) less than 13 (WHO grade IV and V malnutrition gradation) [9], hospitalized for a enteral nutritional support. Exclusion criteria included: prescribed antibiotics in the two months preceding hospitalization, the presence of a disease that may be associated with a change in microbiota profile (diabetes, digestive pathology, metabolic disease), history of obesity, inflammatory and/or autoimmune disease before the onset of anorexia nervosa. Patients hospitalized in compulsory care or legal proceedings were also excluded.

2.3.2. Controls

Healthy normal weight (BMI between 18.5 and 25) volunteers were enrolled consecutively after a medical interview, among hospital's medical and paramedical staff. Past and actual overweight (BMI $\geq$ 25) volunteers were excluded because of a potential presence of gut microbiota dysbiosis [10]. Healthy controls should not have received antibiotics in the two months prior inclusion. They had neither metabolic abnormalities, nor chronic and/or acute disease.

2.4. Clinical and biological phenotyping

AN patients underwent a structured interview performed by a physician, the following variables were collected: age, sex, AN type (restricting versus binge-eating-purging types) according to DSM-IVr criteria and duration of illness. The same operator, measured for each patient, height and weight under standardized conditions, in the morning, after 12 hours fasting period, in light clothes. Body mass index (BMI) was calculated as body weight divided by squared height.

Evaluation of FIDs intensity was carried out by each patient through the "Francis score"; a self-administered questionnaire, which quantifies the severity of functional intestinal disorders (validated score for IBS) and its impact on quality of life. Francis score includes two items to assess the presence of abdominal pain and bloating. Four visual analog scales assess pain intensity, bloating, impact on quality of life and patient intestinal transit. Finally, it provides the number of days with pain over the last ten days. The score ranges from 0 (no symptoms) to 500 (maximum severity), and scores between 75 and 174 symptoms, 175–229 symptoms, and 230–299, define mild, moderately severe and severe symptoms respectively, whereas a score greater than 300 defines very severe symptoms [11].

Stool shape and consistency were determined using the Bristol scale (type 1 through type 7) [12]. The same eating disorder experienced dietitian carried out a quantitative and qualitative dietary investigation of the 48 hours prior the stool collection. Total oral and enteral calories, proteins, carbohydrates and lipids, as well as fiber intakes were captured.

For each patient, a standard biological and nutritional assessment was performed including blood count, blood electrolytes, urea, creatinin, phosphoremia, calcemia, magnesaemia, liver function tests (AST, ALT, Gamma GT, alkaline phosphatase), total bilirubin, hemostasis, lipid balance (total cholesterol, LDLc, HDLc, triglycerides), albumin, transthyretin, CRP, fibrinogen, vitamins (B1, folates, B12, 25OH-D3), trace-elements (copper, selenium and zinc), and plasma citrulline level (assayed by Ion exchange chromatography). The circulating citrulline, a functional mass of enterocytes marker [13], is an amino acid synthesized by the enterocytes then released into the circulation. Biological parameters were not recorded for healthy control subjects.

2.4.1. Stool sample and fecal DNA extraction

One fecal sample (approximately 2 g) was collected for each patient and each control. Fecal collection and DNA extraction were performed as previously described, following Standard Operating Procedure 07 of the IHMS (microbiome-standards.com).

2.4.2. Sequencing of the gene encoding the 16S ribosomal RNA

After DNA extraction, the dominant microbial diversity was determined for each sample by PCR amplification of a region of the gene encoding the 16S rRNA comprising the hyper variable regions V3 and V4. Modified primers of Klindworth et al., 2013 [14] were used, following a standardized and optimized preparation protocol (Metabiote®, GenoScreen, Lille, France). PCR was performed using 5 ng of genomic DNA, using 55 bar coded primers at the final concentration of 0.2 μM and an annealing temperature of 50 °C for 30 cycles (Metabiote® MiSeq primers, GenoScreen, Lille, France). The final libraries of 16S-amplicons were cleaned with a home-made protocol using magnetic beads, quantified and multiplexed at equal concentration. Sequencing was carried out using a protocol for sequencing paired ends on the IlluminaMiSeq platform (Illumina, San Diego, USA) at GenoScreen, Lille, France. The raw readings of base pairs were subjected to the following process: 1) quality filtering with PERL PRINSEQ-lite script by truncation of 3′end bases, with REDSP quality score <30; 2) assembly of the reads using FLASH [15], with an overlap of at least 30 bases and 9%

overlap identity; 3) search and removal of forward and reverse primers using CutAdapt, with no mismatch allowed for primer sequences. Complete 16S sequences for which forward and reverse primers were not found have been eliminated.

2.5. Sequence data analysis

We loaded the sequences into the QIIME 1.9.1 pipeline for analysis, as described by Navas-Molina et al. [16]. From 55 fecal samples we obtained a total of 1.63 million of high-quality sequences with an average read length of 400 nucleotides.

We used the UCLUST algorithm to cluster filtered sequences into operational taxonomic units (OTUs) or molecular species based on a 97% similarity threshold [16]. Then, we identified and removed chimeric sequences using ChimeraSlayer [17]. Since each OTU can comprise many related sequences, we picked a representative sequence from each one. Representative sequences were aligned using PyNAST against Greengenes template alignment (gg_13_8 release), and taxonomy was assigned to the detected OTUs using the basic local alignment search tool (BLAST) reference database and the Greengenes/Patric taxonomy-mapping file. The script make_phylogeny.py was used to create phylogenetic trees using the FastTree program [18]. To correctly define species richness for the analysis of between-sample diversity, known as beta diversity, the OTU table was rarefied at 18008 sequences per sample. Rarefaction is used as a normalization procedure to overcome cases in which read counts were not similar in numbers between samples. In order to avoid false positive taxa, OTUs that did not represent at least 0.2% of sequences for any given sample were removed from the resulting OTU table. The summarized_taxa feature was used to classify taxa from the Domain to the Species level. To provide community alpha diversity estimates, we calculated Chao1 and Shannon indexes. To calculate between-sample diversity, weighted (accounting for abundance of observed taxa) and unweighted (accounting for presence/absence of observed taxa) UniFrac metrics were applied to build phylogenetic distance matrices, which were then used to construct hierarchical cluster trees using PCoA representations (Principal Coordinate Analysis)."

2.6. Statistical and correlation analyses

Statistical analyses were carried out in QIIME and in R environment. To work with normalized data, we analyzed an equal number of sequences from both healthy and patient groups. The Kruskal–Wallis one way analysis of variance, a non-parametric test, was used to compare the mean number of sequences of the groups, i.e. that is healthy controls and patients with AN at various taxonomic levels. Since we used nonparametric correlations, significance was determined through permutations. The analysis provided false discovery rate (FDR) corrected P-values (q).

Non-parametric Spearman's correlation coefficient was used to calculate possible relationships among microbial genera and clinical data variables.

3. Results

3.1. Characteristics of patients and controls

Thirty-three patients were eligible; 33 female patients (no men hospitalized in our unit during study period) participated to the study and were compared to 22 healthy volunteers (including one man) (Fig. 1). The main characteristics of patients and controls are presented in Table 1 and Table 2. Weight and BMI were significantly lower in patients compared to healthy controls (30 ± 4 kg vs 59 ± 9 kg, $p = 0.01$; 11.7 ± 1.5 vs 21 ± 2, $p = 0.001$) (Student's t-test).

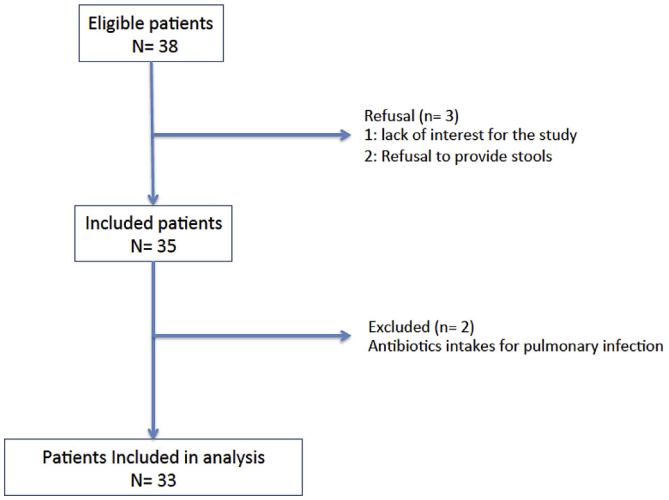

Fig. 1. Flow chart.

Functional intestinal disorders were also significantly more important in patients ($p < 0.001$) compared to controls.

3.2. Nutritional management

At time of stool specimen collection, all the 33 patients were receiving enteral nutrition, since 10 ± 5 days. The same low fiber (<2%), isocaloric isoprotidic solute (1 ml = 1 Kcal) was used.

Table 1
Anthropometric characteristics of patients and controls.

	Patients (n = 33)	Controls (n = 22)	p
Age (year)	32 ± 12	36 ± 12	–
Sex (F/M)	33F/0M	22F/1M	–
Height (cm)	159 ± 10	166 ± 10	–
Weight (kg)	30 ± 4	59 ± 9	0.001
BMI (W/H^2)	11.7 ± 1.5	21 ± 2	0.001
Francis Score (/500)	152 ± 99	25 ± 36	0.000

Table 2
Patients clinical and biological characteristics.

Patients	N = 33	Normal values
BMI	11.7 + 1.5	18.5–24.9
AN Types:		
Restricting type	22 (67%)	–
Purging type	11 (33%)	–
Years of disease	12 ± 11	
Body Composition (DEXA)		
FFM (kg)	26 + 6	–
FM (%)	10 ± 9	–
FFMI	10.3 ± 1.4	–
Albumin (g/l)	36 ± 6	39–48
Transthyretin (mg/l)	0.220 ± 0.116	0.232 ± 0.152
CRP (mg/l)	7 ± 27	<0.5
BNP (ng/l)	66 ± 90	<100
Citrulline (μmol/L)	35 + 14	30–50
Patients with hypertransaminasemia (UI/L)		
AST and/or ALT > 2N	11 (32%)	15–37
AST and/or ALT > 10N	2 (6%)	14–59
Selenium (μ mol/l)	1.2 ± 0.3	0.90–1.50
Zinc (μ mol/l)	11 + 4	12.5–18
Copper (μ mol/l)	14 + 3	0.81–1.41
25OH D3 (ng/ml)	34 ± 16	30–100
Thiamin (nmol/l/l)	668 ± 1402	126–250

Values are number (%) or mean + SD. FFM: Free fat mass; FM: Fat Mass; FFMI: Free Fat Mass Index; BNP: B-type Natriuretic Peptide; CRP: C-Reactive Protein; AST: aspartate transaminases; ALT: alanine transaminase.

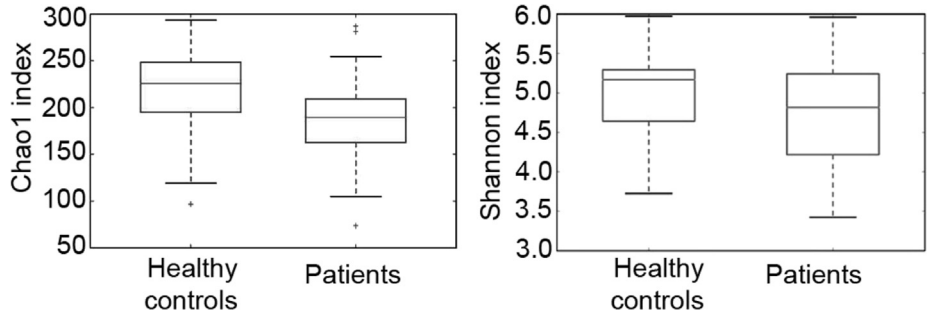

Fig. 2. Alpha-diversity of the faecal microbiome of healthy controls and patients with anorexia based on the Chao1 and Shannon indexes. The microbiome of patients with AN presented significantly lower alpha-diversity when taking into account only the presence/absence of observed taxa ($p = 0.037$ for the Chao1 indexes, Student's t-test) but not when taking into account abundance of each observed taxa ($p = 0.203$ for the Shannon indexes, Student's t-test).

Spontaneous daily oral intakes were less than 25%. Average daily oral and enteral caloric intakes were 1850 Kcal, with 55% carbohydrates, 32% lipids and 12% protein. These intakes were very close quantitatively and qualitatively to the Recommended Nutritional Intakes according to the French National Agency for Food Safety (ANSES 2007), but with the particularity that most of the needs were covered by enteral nutrition through naso-gastric intubation.

3.3. Comparison of microbiota between patients and controls

Patients gut microbiota showed a lower alpha-diversity when accounting for presence/absence of taxa observed ($p = 0.03$ for Chao1 indexes) and but not when accounting for abundance of each observed taxa ($p = 0.203$ for Shannon indexes) compared to controls (Fig. 2). Differences found between fecal microbiota components of patients and healthy controls were also shown by the principal coordinate analysis (Fig. 3). Microorganisms belonging to the genera *Eubacterium, Roseburia, Anaerostipes and Peptostreptococcaceae* (strictly anaerobic Gram positive bacteria) were significantly more abundant in controls, while *Turicibacter, Anaerotruncus, Salmonella and Klebsiella* were significantly more abundant in patients (Fig. 4).

3.4. Relationship between intestinal gut microbiota and functional intestinal disorders (Table 3)

Patients with functional intestinal disorders evaluated by Francis score had a lower abundance of an unknown genus belonging to *Peptostreptococcaceae* family ($r = -0.581$; $p = 0.002$) and increased abundance of *Dialister* ($r = 0.392$, $p = 0.047$), *Robinsoniella* ($r = 0.444$; $p = 0.023$) and *Enterococcus* ($r = 0.488$; $p = 0.011$) (Table 3). There was no other significant clinical or biological difference between the two groups.

3.5. Relationship between the intestinal microbiota and bio–clinical parameters of patients (Table 3)

3.5.1. Undernutrition(BMI)

The severity of undernutrition expressed by BMI was negatively correlated with the Verrucomicrobiaceae ($r = -0.307$; $p = 0.025$) and Ruminococcacea families ($r = -0.456$; $p = 0.001$) and positively with the Clostridiales order ($r = 0.340$; $p = 0.013$), Turicibacteraceae ($r = 0.390$; $p = 0.004$) and Eubacteriaceae families ($r = 0.407$; $p = 0.002$).

3.5.2. The enterocytic function (Citrulline)

Alteration of the enterocytic function expressed by low blood citrulline was correlated with a high abundance of Flavobacteriaceae ($r = -0.379$; $p = 0.036$) and low abundance of Streptococcaceae ($r = 0.432$; $p = 0.015$) and Lachnospiraceae ($r = 0.444$; $p = 0.023$).

3.5.3. Liver enzymes

Hypertransaminasemia, defined as an increase in AST and/or ALT of more than twice the normal value, was correlated negatively with Desulfovibrionaceae ($r = -0.403$; $p = 0.022$) and positively

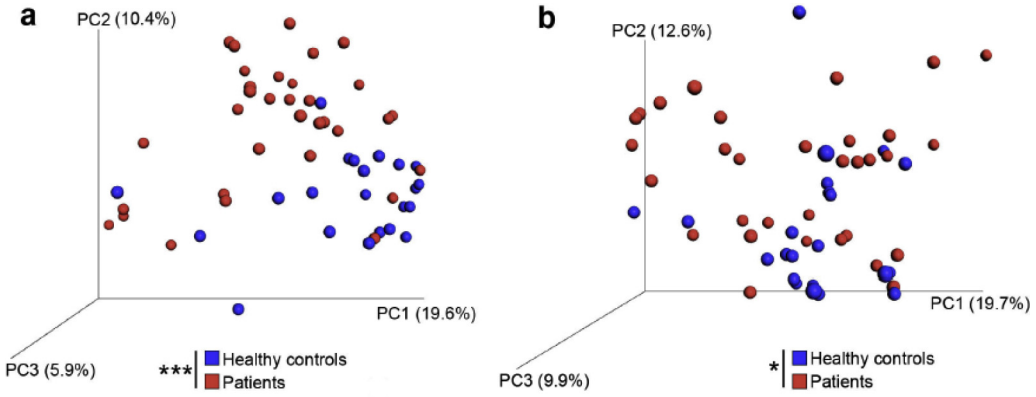

Fig. 3. Unweighted (a) and weighted (b) UniFrac Principal Coordinate Analysis representation of the two groups of subjects (healthy controls and patients with AN). Significant differences were found between healthy controls and AN patients (NPMANOVA test, $^{***}p < 0.001$ for the unweighted and $^{*}p < 0.05$ for the weighted UniFrac PCOA). NPMANOVA = non-parametric multivariate analysis of variance.

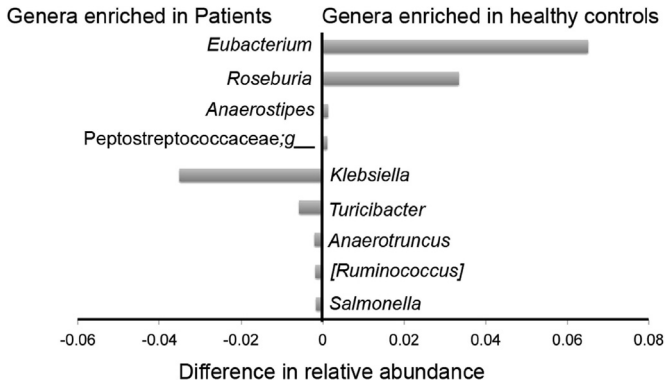

Fig. 4. Taxonomic differences were detected between healthy controls and AN patients using the Kruskal–Wallis test (corrected p values; false discovery rate <0.05). Only genera with significant differences in the relative abundance between healthy controls and patients were displayed.

with Flavobacteriaceae (r = 0.365, $p = 0.04$), Coriobacteriaceae (r = 0.379; $p = 0.032$) and Turicibacteraceae (r = 0.373; $p = 0.035$).

3.5.4. Alimentary intakes (macronutrients)

The mean carbohydrate intake of the patients was positively correlated with the presence of Lachnospiraceae (r = 0.374; $p = 0.035$), while fiber intake was negatively correlated with the proportion of Clostridiales Family XI Incertae Sedis (r = 0.466; $p = 0.007$). No correlation was found with the intake of proteins.

4. Discussion

This study reported an intestinal microbiota dysbiosis in severely malnourished AN female patients undergoing enteral nutrition, compared to healthy controls. This dysbiosis was characterized by a decrease in the microbial richness and a trend towards a decrease in microbial diversity. The relative abundance of the *Klebsiella, Turicibacter, Anaerotruncus, Ruminococcus* and *Salmonella* genera was significantly higher in patients; while the relative abundance of *Eubacterium, Roseburia, Anaerostipes* and Peptostreptococcaceae was significantly higher in controls. These abundance trends illustrate an altered host-microbes symbiosis with an increase of pro-inflammatory bacteria on one hand; a concomitant decrease in usual symbionts of the Firmicutes phylum on the other hand considered as protective via production of short chain fatty acids such as butyrate (*Eubacterium* and *Roseburia* among these).

Dysbiosis was significantly correlated with FIDs intensity with an increase in the relative abundance of *Enterococcus, Robinsoniella and Dialister genera* and a decrease in the abundance of an unknown genus from the Peptostreptococcaceae family. Although Peptostreptococcaceae, *Dialister*, *Robinsoniella* and *Enterococcus* appears as an interesting biomarkers associated with the severity of functional bowel disorders, little is known about the observed taxa relation to a mechanistic contribution to symptoms or their alleviation. *Dialister* and *Enterococcus* have been associated with IBS or SIBO respectively [19], but there are conflicting observations between species, especially for the genus *Enterococcus* [20]. Studies including mechanistic aspects are needed to clarify these interesting outcomes. FIDs associated symptoms reduced microbial richness, these results were consistent with recently published observations in IBS patients. Beyond the FIDs, dysbiosis was also significantly correlated with the severity of undernutrition (BMI), hypertransaminasemia and alteration of enterocyte function (citrulline).

The qualitative and quantitative dietary intakes of patients were modified compared to controls. Fibers and Carbohydrates intakes were higher than in controls. Level of fibers is inversely correlated with relative abundance of *Finegoldia*, while carbohydrate intake is correlated with relative abundance of *Blautia*. Impact of carbohydrates and fibers on human gut microbiota have previously been suggested [21].

Intestinal microbiota implication on the physiopathological mechanisms of undernutrition has already been explored in malnourished children, showing a decrease in microbial diversity [22].

Morita C et al., using the 16S rRNA PCR technique, reported a lower microbial richness and diversity in AN malnourished patients compared to healthy controls [23]. An other recent study confirms these outcomes [24]. Our study outcomes showed a lower alpha-diversity in terms of richness but not when taking into account

Table 3
Correlations between IM dysbiosis, intensity of FID and clinical and biological parameters. (c = class, f = family; g = genus). Only bacterial taxa showing significant correlations with physio-pathological variables are indicated.

	Microbien groups	Corrélation (r)	P value
Francis score	Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus	0.488	0.011
	Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Robinsoniella	0.444	0.023
	Firmicutes;c__Negativicutes;o__Selenomonadales;f__Veillonellaceae;g__Dialister	0.392	0.047
	Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	−0.581	0.002
BMI	Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;g__Eubacterium	0.407	0.002
	Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter	0.390	0.004
	Tenericutes;c__Mollicutes;o__RF39;f__;g__	0.341	0.013
	Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.340	0.013
	Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia	−0.307	0.025
	Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Anaerotruncus	−0.456	0.001
CITRULINE	Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	0.432	0.015
	Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes	0.380	0.035
	Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Robiginitalea	−0.379	0.036
AST	Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.379	0.032
	Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Robiginitalea	0.365	0.040
	Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	−0.403	0.022
ALT	Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter	0.373	0.035
	Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Robiginitalea	0.372	0.036
	Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	−0.405	0.022
B12	Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella	0.377	0.033
Fibres	Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiales Family XI. Incertae Sedis;g__Finegoldia	−0.466	0.007
Carboydrates	Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia	0.374	0.035

richness and evenness in patients compared to healthy controls; maybe because our patients were refeeded with enteral nutrition which may explain the return to gut microbial normal structure without modifying its composition. Enteral nutritional intakes could play a role in bacterial level recovery in severely malnourished patients with AN and opens perspectives of targeted microbiota modulation, by adaptation of nutritional intake during enteral renutrition in order to prevent and/or treat FIDs. The low gut microbial richness seems to persist despite enteral nutrition set up 10 days in average prior stool collection and raises the question of the anteriority of this dysbiosis to weight loss or circular causalities (vicious circle) as suggested above. Indeed, in our clinical experience, AN malnourished patients often describe FIDs as a triggering factor of food restriction. Another study with a higher number of patients (55 patients vs 55 normal weight controls) did not find a significant beta-diversity difference between the intestinal microbiota of AN patients and controls; patients were younger and less undernourished (mean BMI 15.3 ± 1.4); weight gain, however, resulted in a significant increase in the number of OTUs compared to controls [25]. Finally, dysbiosis may also be influenced by the severity of malnutrition and AN/or duration.

Gram-negative bacteria (GNB) of the genus *Klebsiella* and *Salmonella* were more abundant in patients. These latter two genera belong to the Enterobacteriaceae family and known to have a high pro-inflammatory and pathogenic potential [26] [27]. In patients with AN, more or less severe sepsis episodes with GNB are described [28]. Further studies on associated factors to this dysbiosis could help identifying patients who will likely develop this type of infectious complications in order to better prevent the associated vital risks.

Two bacterial genera (*Eubacterium and Roseburia*) belonging to the Firmicutes phylum are much less abundant in the patients. These bacteria are among the dominant producers of butyrate, a short chain carboxylic acid that plays an important role in the immuno-regulatory processes at the level of the intestinal mucosa [29]. These results suggest a potential immune weakening in these AN patients.

Finally, our study results confirmed for the first time our initial hypothesis of an association between the severity of FIDs and the presence of intestinal microbiota dysbiosis.

Other associations found between dysbiosis and either severity of malnutrition (BMI), enterocytic involvement (Citrulline) and hypertransaminasemia raises questions about the role of microbiota in the perpetuation of undernutrition and the susceptibility to develop metabolic complications.

4.1. Limitations

This study presents several limitations; the first is the small size of the studied population, despite major alterations of the host-microbe symbiosis. Second, there is no gut microbiota dysbiosis follow up after weight gain. Third, the 16S rRNA method covers only bacteria and does not allow taking into account viruses and fungi. It also only gathers taxonomic information when an in-depth metagenomic study would indicate potential links with the overall functions of the microbiota and their implication in the onset or chronicization of FIDs and AN.

5. Conclusions

FIDs are frequently described in AN patients and the present study shows a strong correlation between gut microbiota dysbiosis and FIDs severity. In addition, gut micriobiota dysbiosis was correlated with the severity of undernutrition, hypertransaminasemia and enterocyte function.

These outcomes support the concept of an altered host-microbes symbiosis that could be one of the key factors in the onset and/or chronicity of AN. Dysbiosis evolution after weight recovery needs further investigations.

Finally, these results open the door to further metagenomic and translational studies to explore and assess either links between microbial genes, with their functions, and predictive biomarkers, and also the design and validation of nutritional and biotherapeutic strategies that aims at restoring a balanced host-microbes symbiosis.

Author contributions

The authors' responsibilities were as follows: MH performed the study design, conducted the study and wrote the article with the support of all co-authors; CM carried out all the statistical analyses, and was the major contributor to methodology and results section; ASr and VP Both contributed to the statistical analyses and the whole article review; FL contributed to writing the result section and review the manuscript final version; NC, dietician in our unit, carried out the food surveys of patients and controls; JD: contributed to the emergence of the initial idea of the existence of a Gut dysbiosis in anorexia nervosa patients and to the elaboration of the study design. He alsowas a major contributor of the article writing; J-CM contributed significantly to the study design, its implementation and follow up. He also was a major contributor of the article writing and review.

Sources of support

This study was supported by the Instituto de Salud Carlos III/FEDER (PI17/00614).

Conflict of interest

We declare no conflict of interest.

Acknowledgements

We thank DOMINGO Nabila-Yasmine and all Clinical Research Facility staff of Raymond Poincare University hospital excellent assistance during the studies.

References

[1] Brown C, Mehler PS. Medical complications of anorexia nervosa and their treatments: an update on some critical aspects. Eat Weight Disord EWD déc 2015;20(4):419–25.
[2] Roux H, Chapelon E, Godart N. Epidemiology of anorexia nervosa: a review. L'Encéphale avr 2013;39(2):85–93.
[3] Eddy KT, Dorer DJ, Franko DL, Tahilani K, Thompson-Brenner H, Herzog DB. Diagnostic crossover in anorexia nervosa and bulimia nervosa: implications for DSM-V. Am J Psychiatry 2008 Feb;165(2):245–50. https://doi.org/10.1176/appi.ajp.2007.07060951 [Epub 2008 Jan 15].
[4] Chiarioni G, Bassotti G, Monsignori A, Menegotti M, Salandini L, Di Matteo G, et al. Anorectal dysfunction in constipated women with anorexia nervosa. Mayo Clin Proc oct 2000;75(10):1015–9.
[5] Sileri P, Franceschilli L, De Lorenzo A, Mezzani B, Todisco P, Giorgi F, et al. Defecatory disorders in anorexia nervosa: a clinical study. Tech Coloproctol mai 2014;18(5):439–44.
[6] Wang X, Luscombe GM, Boyd C, Kellow J, Abraham S. Functional gastrointestinal disorders in eating disorder patients: altered distribution and predictors using ROME III compared to ROME II criteria. World J Gastroenterol 21 nov 2014;20(43):16293–9.
[7] Ringel Y, Ringel-Kulka T. The intestinal microbiota and irritable bowel syndrome. J Clin Gastroenterol déc 2015;49(Suppl. 1):S56–9.
[8] Jeffery IB, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EMM, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut juill 2012;61(7):997–1006.
[9] Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995;854:1–452.

[10] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 21 déc 2006;444(7122):1027–31.
[11] Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther avr 1997;11(2):395–402.
[12] Riegler G, Esposito I. Bristol scale stool form. A still valid help in medical practice and clinical research. Tech Coloproctol déc 2001;5(3):163–4.
[13] Crenn P, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B. Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. Gastroenterology mai 2003;124(5):1210–9.
[14] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 7 janv 2013;41(1):e1.
[15] Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinf Oxf Engl 1 nov 2011;27(21):2957–63.
[16] Navas-Molina JA, Peralta-Sánchez JM, González A, McMurdie PJ, Vázquez-Baeza Y, Xu Z, et al. Advancing our understanding of the human microbiome using QIIME. Methods Enzymol 2013;531:371–444.
[17] Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res mars 2011;21(3):494–504.
[18] Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol juill 2009;26(7):1641–50.
[19] Lopetuso LR, Petito V, Graziani C, Schiavoni E, Paroni Sterbini F, Poscia A, et al. Gut microbiota in health, diverticular disease, irritable bowel syndrome, and inflammatory bowel diseases: time for microbial marker of gastrointestinal disorders. Dig Dis Basel Switz 2018;36(1):56–65.
[20] Pyleris E, Giamarellos-Bourboulis EJ, Tzivras D, Koussoulas V, Barbatzas C, Pimentel M. The prevalence of overgrowth by aerobic bacteria in the small intestine by small bowel culture: relationship with irritable bowel syndrome. Dig Dis Sci mai 2012;57(5):1321–9.
[21] Klinder A, Shen Q, Heppel S, Lovegrove JA, Rowland I, Tuohy KM. Impact of increasing fruit and vegetables and flavonoid intake on the human gut microbiota. Food Funct avr 2016;7(4):1788–96.
[22] Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 1 févr 2013;339(6119):548–54.
[23] Morita C, Tsuji H, Hata T, Gondo M, Takakura S, Kawai K, et al. Gut dysbiosis in patients with anorexia nervosa. PloS One 18 déc 2015;10(12), e0145274.
[24] Borgo F, Riva A, Benetti A, Casiraghi MC, Bertelli S, Garbossa S, et al. Microbiota in anorexia nervosa: the triangle between bacterial species, metabolites and psychological tests. PloS One 2017;12(6), e0179739.
[25] Mack I, Cuntz U, Grämer C, Niedermaier S, Pohl C, Schwiertz A, et al. Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints. Sci Rep [Internet]. 27 mai 2016 [cité 4 juill 2017];6. Disponible sur: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4882621/.
[26] Hsu C-R, Pan Y-J, Liu J-Y, Chen C-T, Lin T-L, Wang J-T. Klebsiella pneumoniae translocates across the intestinal epithelium via Rho GTPase- and phosphatidylinositol 3-kinase/Akt-dependent cell invasion. Infect Immun févr 2015;83(2):769–79.
[27] Jeziorski E, Ludwig C, Rodière M, Marchandin H, Vande Perre P. Gut translocation septicemia in infant. Arch Pediatr Organe Off Soc Francaise Pediatr sept 2010;17(Suppl. 4):S179–83.
[28] Mattinson A, Pelosi AJ, Jamieson A. Spontaneous bacterial peritonitis in a patient with anorexia nervosa with profound zinc and iron deficiency. Am J Med août 2015;128(8):e1–2.
[29] Nepelska M, Cultrone A, Béguet-Crespel F, Le Roux K, Doré J, Arulampalam V, et al. Butyrate produced by commensal bacteria potentiates phorbol esters induced AP-1 response in human intestinal epithelial cells. PloS One 2012;7(12), e52869.