Posters & Abstracts

2019

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New test for profiling gut microbiota in IBS, non-IBD and IBD patients

Vebø H[1], Casén C[1], Kreso S[1], Vangen DL[1], Ricanek P[2], Perminov G[3], Sauar J[6], Halvorsen FA[7], Røseth A[8], Vatn MH[4,5]

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DDW Orlando May 19, 2013. Poster ID: Su1185

BACKGROUND

The increasing awareness of the gut microbiota's effect on our health has triggered the need for tools to monitor these microbes. The GA-map™ technology platform has been developed to demonstrate profiles of the composition of the gut microbiota. The platform provides analysis of a large number of faecal samples in a time- and cost effective way.

In this study, fecal samples from patients diagnosed with IBS and normal control subjects have been compared using The GA-map™ microbiota test. In addition, a sub-cohort of IBD and non-IBD patients have been analyzed.

METHODS AND STUDY COHORTS

Table 1. Overview of study cohorts

Cohort	N	Diagnosing	Sample matrix	Origin
IBS	31	Rome III criteria Colonoscopy and/or Faecal Calprotectin	DNA stabilizing STAR buffer (Roche Inc.)	Norwegian multi-center study*
IBD	105	Colonoscopy and/or Faecal Calprotectin	Frozen native	IBSEN II study (2005-2007)[1,2]
non-IBD	82	Colonoscopy and/or Faecal Calprotectin	Frozen native	IBSEN II study (2005-2007)[1,2]
Control	78	No clinical signs of GI disorder. Inclusion by MD interview	DNA stabilizing STAR buffer (Roche Inc.)	Working places in Oslo area

**Sykehuset Telemark HF, Vestre Viken HF, Lovisenberg Diakonale sykehus*

The GA-map™ microbiota test

Based on peer-reviewed literature and our own research, unic
to facilitate separation between patient groups and control su
Total bacterial DNA were extracted from the faecal samples a
processed on the GA-map™ platform, as described in[3].

RESULTS

Table 2. Results from GA microbiota PLS-DA analysis of diagnosed IBS patients vs healthy control subjects, and IBD vs non-IBD patients.

Cohort	N	Sensitivity*	Specificity*
IBS vs controls	31/78	87 %	77 %
IBD/non-IBD	105/82	78 %	70 %

**Leave-one-out validation, not an independent patient population*

Illustration of bacterial profiles

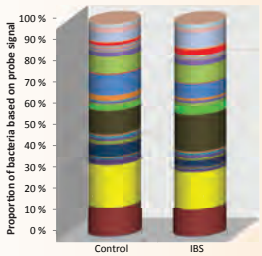

Figure 1. Bacterial profiles of the average of the IBS and control sample populations

Increase and decrease in bacteria related to IBD

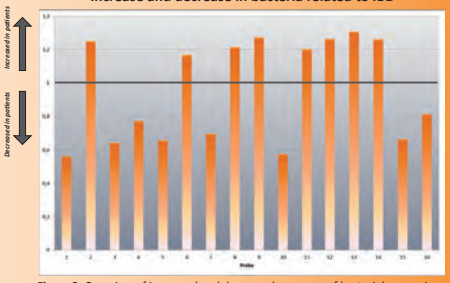

Figure 2. Overview of increased and decreased presence of bacterial groups in fecal samples from IBD patients (n=105) as compared to the non-IBD patients (n=82). Value 1 on the Y-axis represents the non-IBD patients.

CONCLUSION

The GA-map™ microbiota test gives a unique opportunity to study specific profiles of the gut microbiota that may be associated with GI related disorders. The results suggest that the GA test may be a useful tool in differentiating between IBS and control subjects, and IBD/non-IBD patients, and thus an aid in the diagnosis and follow up of patients with inflammatory and functional GI disorders.

9th Congress of ECCO-IBD, February 20-22, 2014, Copenhagen, Denmark **Poster presentation: Po42**

Microbiota stability *in vivo* and *in vitro*

Hegge, F.T.[1], Vebo, H.[1], Ciemniejewska, E.[1], Froyland, C.[1], Kreso, S.[1], Sekelja, M.[1], Roseth, A.[2], Casen, C.[1]

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...ek P, Brackmann S, Perminow G, Lyckander LG, Sjoetheim J, Holme O, Hale O, Rydning A, Vatn MH; IBSEN II Study Group. 2011. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. Scand J Gastroenterol. ...o;46(9):1081-91. Epub 2011 May 30.
...now G, Brackmann S, Lyckander LG, Franke A, Boethne A, Rydning A, Aamodt G, Schreiber S, Vatn MH; IBSEN-II Group. 2009. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005-07, ...increased incidence in Crohn's disease. Scand J Gastroenterol. 2009;44(4):446-56.
...HC, Sekelja M, Nesbekkg R, Storrø O, Johnsen R, Øien T, Rudi K. 2013. Temporal development of the infant gut microbiota in immunoglobulin E-sensitized and non-sensitized children determined by the GA-map infant array. Clin Vaccine Immunol. Aug;18(8):1226-35. ...11 Jun 8.

New test for profiling of the gut microbiota in non-IBD and IBD patients

Heidi Vebø[1], Dina Lilleseth Vangen[1], Finn Terje Hegge[1], Christina Casen[1], Petr Ricanek[2], Gøri Perminow[3], Morten H. Vatn[4,5]
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Genetic Analysis
www.genetic-analysis.com

BACKGROUND

As the awareness of the effect of the gut microbiota on health is increasing, there is a need to find tools to study variances and changes in the gut microbiota. GA is developing a 16s rRNA DNA based test using the GA-map™ technology platform to demonstrate profiles of the composition of gut microbiota in a cost-effective way.

Scientific publications on the gut microbiota

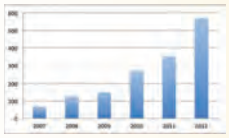


Figure 1: *Medline search on "gut microbiota" in Title/Abstract*

A sub-cohort from the IBSEN II study cohort has been analysed together with a population of normal subjects on the GA test to demonstrate the test's ability to discriminate the gut microbiota of IBD and non-IBD patients, and normal subjects.

MATERIALS AND METHODS

The Product
Based on peer-reviewed literature and a test optimizing development work, special sets of DNA probes are designed that facilitate bacterial profile separation between the patient groups IBD and non-IBD, and normal subjects.

The IBSEN II cohort
The samples used were aliquots from frozen faecal samples collected from patients recruited for the prospective Inflammatory Bowel Disease South-Eastern Norway (IBSEN) II study (2005-2007)1,2, including treatment naïve IBD patients (105) and symptomatic non-IBD (82) patients. The samples were collected before colonoscopy.

The normal population
In addition a population of 84 normal subjects with no clinical signs or symptoms of gut disorder (not confirmed by colonoscopy) was included. The normal subjects was recruited from working places in the Oslo Area and had passed certain inclusion and exclusion criteria after interview by a medical doctor.

The GA test
The GA test was performed essentially as described in (3), using Luminex MagPix system for detection and quantification of labeled DNA probes (indicative of presence of different bacteria).

RESULTS

Total bacterial DNA was extracted from the faecal samples and further processed in the GA-map™ platform. Below the results from the study is summarized. Age-group of subjects ranged from 18 to 55 years. An optimal diaggnostic model for prediction of non-IBD and IBD was developed.

	IBD vs non-IBD	IBD vs normal	non-IBD vs normal
Patients n=	105	63	70
Non-IBD/normal n=	82	81	81
Total samples n=	187	144	151
Sensitivity	78%	84%	74%
Specificity	70%	84%	83%
Accuracy	74%	84%	79%
AUC	0.81	0.92	0.87

Table 1: *Summary of GA-map test analysis on clinical samples. The statistical model was built using leave-one-out cross-validation.*

Up- and down regulation of bacteria in faecal samples from IBD patients

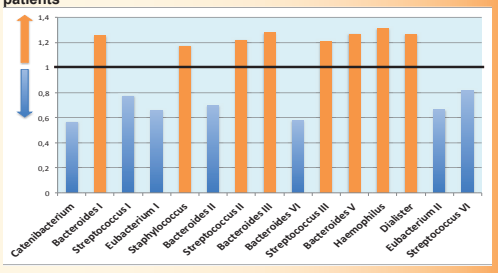
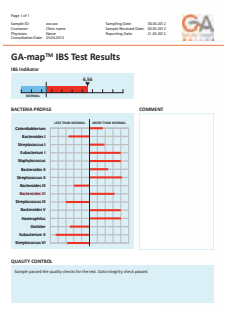


Figure 2: *Patient samples (n=105) compared to the normal population (n=84). Value 1 on the Y-axis represents the normal population.*

Test result report examples

A



B

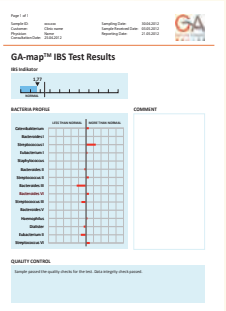


Figure 3: Examples of design of result reports for the GA IBS test. Report A shows a positive test result, while report B shows a negative test result. The test's cut-off is marked as a vertical line on the IBS indicator.

CONCLUSION

The GA test gives a unique opportunity to study specific profiles of the gut microbiota that may be associated with non-IBD and/or IBD related diseases compared to gut microbiota profiles in normal subjects.

The present results suggest that the GA test may be a useful tool in differentiating between IBD, non-IBD and normal subjects, and thus an aid in the diagnosis and follow up of patients.

PUBLICATIONS

1. Ricanek P, Brackmann S, Perminow G, Lyckander LG, Sponheim J, Holme O, Høie O, Rydning A, Vatn MH; IBSEN II Study Group. 2011. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. Scand J Gastroenterol. 2011 Sep;46(9):1081-91. Epub 2011 May 30.

2. Perminow G, Brackmann S, Lyckander LG, Franke A, Borthne A, Rydning A, Aamodt G, Schreiber S, Vatn MH; IBSEN-II Group. 2009. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005-07, showing increased incidence in Crohn's disease. Scand J Gastroenterol. 2009;44(4):446-56.

3. Vebø HC, Sekelja M, Nestestog R, Storrø O, Johnsen R, Øien T, Rudi K: 2011 Temporal development of the infant gut microbiota in immunoglobulin E-sensitized and non-sensitized children determined by the GA-map infant array. Clin Vaccine Immunol. Aug;18(8):1326-35. Epub 2011 Jun 8.

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Microbiota analysis in IBS and IBD/non-IBD patients and normal subjects

UEGW Berlin Oct 15, 2013

Poster ID: P592

www.genet-analysis.com

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[5]Clinical Medicine EpiGen, Campus Ahus, University of Oslo, Norway;

BACKGROUND

The increasing awareness of the gut microbiota effect on our health has triggered the need for tools to monitor these microbes. The GA-map™ technology platform has been developed to display profiles of the composition of the gut microbiota. The platform provides analysis of a large number of faecal samples in a time- and cost effective way.

In a multi center trial, fecal samples from patients diagnosed with IBS have been collected and compared to fecal samples from normal subjects.

In another study a sub-cohort of IBD and non-IBD patient samples have been analyzed.

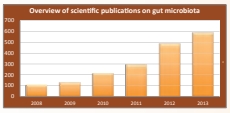

Figure 1: Medline search Oct 2013 on "gut microbiota" in Title/Abstract

MATERIAL AND METHODS

Normal population – Model building

The gut microbiota reference range was defined based on 165 samples from normal subjects with no clinical signs or symptoms of gut disorder (not confirmed by colonoscopy). These subjects were recruited by one Swedish clinical site and from working places in the Oslo Area and had passed certain inclusion and exclusion criteria after interview by a medical doctor.

IBS patients

Patients were recruited in a multi center study involving three Norwegian and one Danish hospital. Patients were diagnosed with IBS according to the Rome III criteria, after exclusion of inflammation by colonoscopy and/or F-calprotectin analysis. In total 127 samples.

IBD and non-IBD patients

The samples used were aliquots collected from patients recruited for the prospective Inflammatory Bowel Disease South-Eastern Norway (IBSEN) II study[1,2], including treatment naïve 105 IBD patients and symptomatic 82 non-IBD patients. The samples were collected before colonoscopy.

Table 1. Study cohorts, collaborators in IBS multi-center trial and fecal sample origins

Cohort	Sample origin	Principal Investigator	N	Recruitment
Normal subjects (n=165)	Karolinska Institute Science Park, Sweeden	Prof. Lars Engstrand	60	Clinical investigation by medical doctor
	Working places in Oslo area	MSc. Christina Casén	105	
IBS patients (n=127)	Herlev University Hospital, Denmark	Prof. Pia Munkholm	64	Rome III criteria, Colonoscopy and F-Calprotectin
	Drammen hospital, Norway	Dr. Fred-Arne Halvorsen	24	
	Telemark Hospital, Norway	Dr. Jostein Sauar	16	
	Lovisenberg Diakonale Hospital, Norway	Dr. Arne Røseth	23	
IBD/ non-IBD (n=187)	IBSEN II study (2005-2007)[1,2], Norway	Prof. Morten Vatn	105/82	Colonoscopy and F-Calprotectin

The establishment of GA bacterial panel

Based on peer-reviewed literature and our own research, special sets of DNA probes were designed to facilitate separation between patient groups and normal subjects based on their bacterial profile[3].

RESULTS AND DISCUSSION

Results from IBS study

Table 2. Validation results using 127 IBS patient samples

IBS patients with dysbiosis Average of sampling sites
71 %

Table 3. Sampling site spesific results:

Sampling site	IBS patients with dybiosis
Lovisenberg Hospital, (Norway)	48 %
Skien Hospital, (Norway)	56 %
Drammen Hospital, (Norway)	79 %
Herlev University Hospital (Denmark)	86 %

Illustration of bacterial profiles

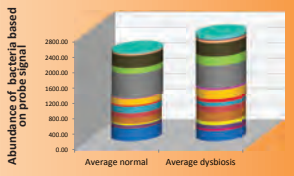

Figure 2. Bacterial profiles of the average of the IBS and control sample populations

The outcome of the clinical validation studies shows that on average 71% of all IBS patients have dysbiosis. These studies show variable frequencies of dysbiosis in different IBS population. According to the principal investigators these differences can be explained by the more severe IBS cases consulted by Herlev University Hospital compared to the smaller local hospitals. In Drammen Hospital, most patients were severe cases, recruited after negative inflammation result from colonoscopy.

Results from IBD study

Increase and decrease in bacteria related to IBD

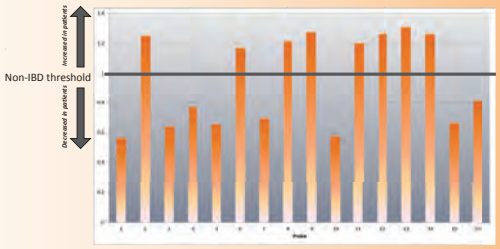

Figure 3. Overview of increased and decreased presence of bacterial groups in fecal samples from IBD patients (n=105) as compared to the non-IBD patients (n=82). Value 1 on the Y-axis represents the non-IBD patients.

CONCLUSION

The GA-map™ Dysbiosis Test can be used to diagnose dysbiosis in IBS patients.
The GA-map™ technology can also be used to differentiate microbiota in IBD/non-IBD patients.
The GA-map™ IBS Dysbiosis Test gives a unique opportunity to study specific profiles of the gut microbiota that may be associated with GI related disorders.

NEXT STEP: Monitoring after treatment

GA is continuing with studies that aim to reveal changes in microbiota before and after treatment of IBS patients. The patients are treated with FODMAP diet and/or supplements and followed for 6 weeks. The dysbiosis status will be measured by the GA-map™ IBS Dysbiosis Test. Another interesting patient group GA is working with is IBD patients in remission, having IBS symptoms. Patients are on treatment for their IBS symptoms , and changes in microbiota before and after treatment are being measured by GA-map™ IBS Dysbiosis Test.

CONTACT DETAILS: Christina Casén M.Sc
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REFERENCES

1. Ricanek P, Brackmann S, Perminow G, Lyckander LG, Sponheim J, Holme O, Høie O, Rydning A, Vatn MH; IBSEN II Study Group. 2011. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. Scand J Gastroenterol. 46:1081-1091
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Abstract sa1245

Gut Microbiota Alterations in IBD Patients with IBS Symptoms Before and After 6 Weeks of Low FODMAP Diet

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Background

Approximately 60-80% of patients with IBD suffer from irritable bowel syndrome (IBS)

Low Fermentable Oligo-Di and Mono-saccharides and Polyols (FODMAP) diet was found to be effective in IBS

Patients with IBD and IBS (IBD – IBS) may have altered microbiota

The aim of this study was to investigate the impact of LFD on fecal microbiota

Patients and Study design

IBD patients in remission, mild to moderate disease activity

Having IBS according to ROME III criteria

Randomized, non-blinded controlled study to
- Low FODMAP diet (LFD) or
- Normal Danish/Western diet (ND)

Diet duration 6 weeks

IBS symptom severity score (IBS-SSS) web-registry at week 0 and 6

IBS-SSS example of web registry

Methods

Bacterial 16SrRNA DNA analysis in fecal samples by GA-map™ Dysbiosis Test (GA)

Dysbiosis status of the gut bacteria was calculated by Dysbiosis index (DI)

DI is calculated by an algorithm based on the bacterial abundance and bacterial profile in a fecal sample in > 100 bacteria

Change in DI between a baseline and a follow-up sample was investigated

GA-map™ Dysbiosis Test some selected bacteria

Results

Characteristics of IBD patients

Treatment	N	Age, median (range)	Gender (female)	CD	UC
All	53	45 (20-69)	81% (45)	25% (13)	75% (40)
LFD	27	41 (20-59)	70% (19)	30% (8)	70% (19)
ND	26	46 (24-69)	92% (24)	19% (5)	81% (21)

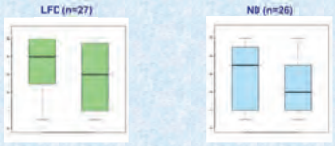
IBS-SSS week 0 vs 6

Treatment	N	IBS-SSS week 0	IBS-SSS week 6	p-value
All	53	210 (60-454)	100 (0-390)	<0.001
LFD	27	210 (90-454)	75 (0-368)	<0.001
ND	26	210 (60-410)	174 (8-300)	<0.01
LFD vs ND				0.05

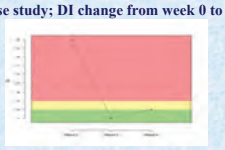
DI status in IBD-IBS: week 0 vs 6

Treatment	N	Week 0		Week 6		p-value
		Dysbiosis	DI (mean)	Dysbiosis	DI (mean)	
All	53	77%	6.7	66%	5.4	<0.01
LFD	27	81%	7.3	70%	6.0	<0.05
ND	26	73%	6.0	62%	4.7	<0.05

Box plot showing significant decrease in DI in LFD and ND groups at week 0 versus week 6



Case study; DI change from week 0 to 6



Conclusion

Both LFD and ND groups reported significant reduction in IBS-SSS from week 0 to 6

High proportions (77%) of the IBD-IBS patients were dysbiotic at baseline

At week 6 more than 50% of the patients showed changes in microbiota

In both LFD and ND groups a significant decrease in dysbiosis index was found after 6 weeks

DDW2014 Digestive Disease Week
May 3-6, 2014
Exhibit Dates: May 4-6, 2014
McCormick Place
Chicago, IL

European Crohn's and Colitis Organisation 9th Congress of ECCO-IBD, February 20-22, 2014, Copenhagen, Denmark **Poster presentation: Po42**

Microbiota stability *in vivo* and *in vitro*

Hegge, F.T.[1], Vebo, H.[1], Ciemniejewska, E.[1], Froyland, C.[1], Kreso, S.[1], Sekelja, M.[1], Roseth, A.[2], Casen, C.[1]
[1]Genetic Analysis AS, Oslo, Norway, [2]Lovisenberg Diakonale Hospital, Oslo, Norway

BACKGROUND

Genetic Analysis AS has developed a proprietary technology for analysis of gut microbiota in humans. The technology enable the simultaneous detection of >100 bacteria using a DNA probe methodology that utilizes common and variable DNA sequences within the 16s rRNA gene – the GA-map™ platform. The technology is currently being commercialized as a gut microbiota dysbiosis test.

Here we present results demonstrating that inter-person differences are larger than the intra-person variation in healthy individuals. Further, we demonstrate that the effect on the microbiota composition of storing a sample for up to 5 days at room temperature is negligible compared to inter-person differences.

CONCLUSIONS

The natural variation occurring in persons living "normal" lives do not influence the results of the GA-map™ IBS dysbiosis, i.e. the inter-person variations dominate the intra-person variations. The microbial fingerprint of each individual is recognizable and specific over time.

The sampling of feces for use in the GA-map™ IBS dysbiosis test can be performed without preservative media up to five days prior to freezing.

MICROBIOTA STABILITY *IN VIVO*

A person's microbiota is expected to exhibit natural variation over time. This variation occur even without the influence of travel, diet or medication (e.g. antibiotics). GA has investigated the normal variation (intra-person variation) in microbiota over multiple sampling points over several months.

METHOD

To accomplish this, 5 volunteers collected samples regularly over a period of more than 2 months. In total 58 samples were collected for this experiment. The volunteers have been living "normal" lives. Any deviations from normal routines was recorded.

RESULTS

As illustrated in figure 1, the inter-person variations dominate the intra-person variations when analysed with the GA-map™ dysbiosis test. Interestingly, volunteer #4 was on antibiotics during the timeperiod when samples d, h and i was collected (orange circle). This is beleived to explain the deviations from the rest of the volunteer 4 samples.

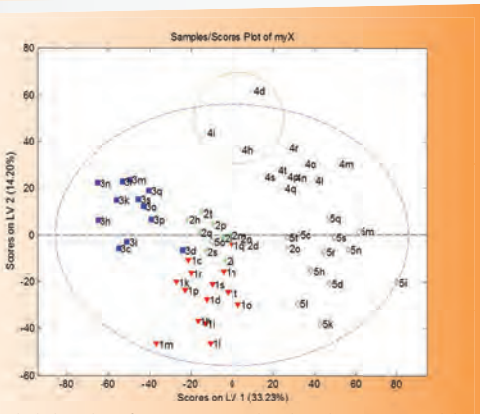


Figure 1. PLS plot displaying the two first components (LV1 and LV2) that explain most variance in dysbiosis. The numbers refer to each individual donator (1-5) and the letters refer to sampling time-point.

Results - normal samples

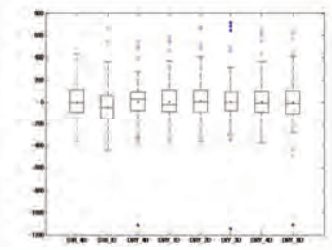


Figure 2. Box plot of PCA scores on log-transformed data from normal samples

Table 1. Storage conditions for normal samples

Sample medium	Storage at RT*
STAR	4 hours
DRY	4 hours
DRY	1 day
DRY	2 days
DRY	3 days
DRY	4 days
DRY	8 days
STAR	8 days

Results - IBS samples

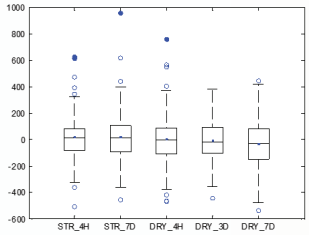


Figure 3. Box plot of PCA scores on log-transformed data

Table 1. Storage conditions for IBS samples

Sample medium	Storage at RT*
STAR	4 hours
DRY	4 hours
DRY	3 days
DRY	7 days
STAR	7 days

**Samples were placed at -20°C until analysis.*

MICROBIOTA STABILITY *IN VITRO*

As faecal samples for commercial testing most often are collected at home by the patient, the sample has to be transported from the collection site to the analysis laboratory. This transportation might take several days, in which the sample is stored in a sample collection tube. During this period both microbial growth and DNA degradation can occur, altering the 16S rRNA gene composition of the sample. GA has investigated how the composition of gut microbiota changes over time in a sample collection tube without any stabilizing buffer.

STAR buffer (Roche Molecular Systems, USA) has been used as reference material in this experiment.

METHOD

10 healthy individuals donated 8 samples (n=80). The samples were storet on STAR buffer or dry-without any preservatives (table 1).

To validate the results a separate study was performed that included samples from 10 patients diagnosed with IBS. Each patient donated 5 samples as outlined in table 2. A total of 50 samples were analysed.

All samples were analysed using the GA-map™ dysbiosis test.

RESULTS

No significant differences (p-value <0.05) was found when using PCA analysis on log-transformed data (figure 2).

The results were validated using IBS samples and no significant differences (p<0.05) between samples were found using PCA analysis on log-transformed data (figure 3).

Genetic Analysis AS - Address: Nycoveien 2, 0482 Oslo, Norway - Phone: +47 48 32 16 10 - e-mail: info@genet-analysis.com - web: **www.genet-analysis.com**

European Crohn's and Colitis Organisation
9th Congress of ECCO-IBD, February 20-22, 2014, Copenhagen, Denmark
Poster no: P474

Gut microbiota alterations in IBD patients with IBS symptoms before and after 6 weeks of low FODMAP diet

Pedersen N, Vinding K, Végh Z, Casen C*, Ankersen D, Carlsen K, Petersen A**, Burisch J, Munkholm P.

Gastroenterology, Herlev University Hospital, Copenhagen, Denmark. Genetic Analysis AS (GA), Oslo, Norway*. Gastroenterology, Hvidovre University Hospital, Copenhagen, Denmark**.

Background

Approximately 60-80% of inflammatory bowel patients (IBD) patients suffer from irritable bowel syndrome (IBS). Low Fermentable Oligo-Di and Mono-saccharides and Polyols (FODMAP) diet (LFD) was found to be effective in IBS patients. Patients with IBD and IBS (IBD – IBS) may have altered microbiota.
The aim of this study was to investigate the impact of LFD on faecal microbiota.

Patients & Study design

IBD patients in remission/mild-moderate disease activity having IBS (ROME III criteria). Randomized, not – blinded controlled study to a LFD or a normal (Western/Danish) diet (ND) during 6 weeks.

Methods

IBS severity score (IBS-SSS) was registered by patients at week 0 and week 6 on an eHealth web-application (Fig 1).
Bacterial 16SrRNA DNA analysis in faecal samples by GA-map™ IBS Dysbiosis Test (GA): A test utilizing DNA probes to recognize deviations from a reference population (dysbiosis) in the most common gut bacteria (Table 1)
Dysbiosis status of the gut bacteria was calculated by Dysbiosis index (DI). Change in DI between a baseline and a follow-up sample was investigated.

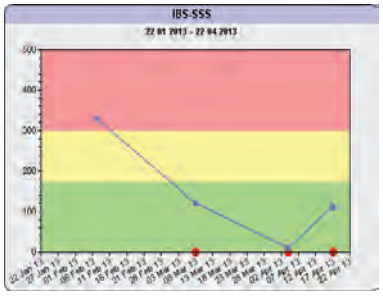


Fig 1. Patient registration of IBS-SSS on www.ibs.constant-care.dk

Table 1. Bacteria for microbiota status in GA test.

Bacteroidetes	**Firmicutes**
Bacteroides	Clostridium
Parabacteroides	Faecalibacterium
Proteobacteria	Eubacterium
Escherichia	Lactobacillus
Pseudomonas	Streptococcus
Actinobacteria	Ruminococcus
Bifidobacterium	**Verucomicrobia**
	Akkermansia

Results

N= 45 IBD-IBS patients
M : F = 1 : 3,3
15 (33%) CD and 30 (67%) UC
Median age 41 years (range 20-69 years)
Treatment groups: 23 (51%) in LFD and 22 (49%) in ND groups

Table 2. IBS-SSS results

	IBS-SSS (range)		
	Week 0	Week 6	p-value
All (N=45)	210 (60-454)	100 (0-368)	<0.001
LFD (N= 23)	210 (90-454	75 (0-368)	<0.0001
ND (N= 22)	210 (60-410)	174 (8-300)	0.001
LFD vs ND			0.05

Table 3. IBD-IBS patients and DI status at treatment week 0 and 6

	Week 0 (N=47)		Week 6 (N=45)		
	Dysbiosis (%)	Normal biosis (%)	Decreased DI (%)	Increase DI (%)	Unchanged DI (%)
All groups	41 (87)	6 (13)	16 (36)	9 (20)	20 (44)
LFD (23)	20 (87)	3 (13)	8 (35)	6 (26)	9 (39)
ND (22)	19 (86)	3 (14)	8 (36)	3 (14)	11 (50)

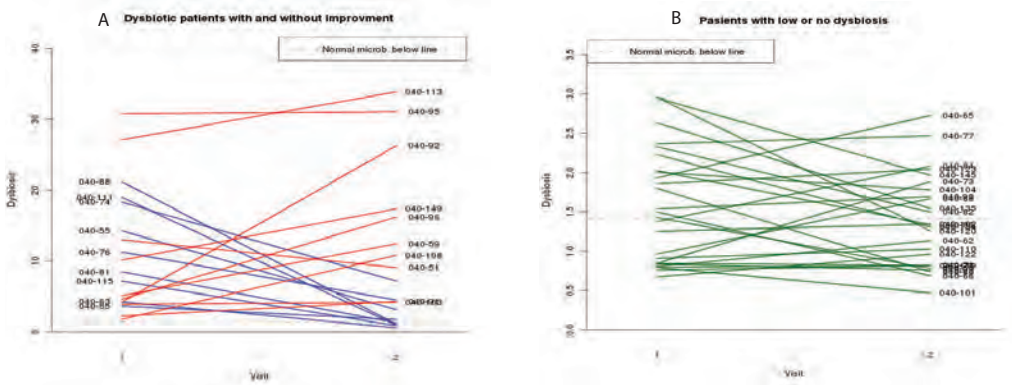


Figure 2. Changes in DI from week 0 (visit 1) to week 6 (visit 2) in patients dysbiotic at week 0 (plot A) and patients with low or no dysbiosis at week 0 (plot B). The blue lines illustrates patients with a decrease in DI, while the red lines illustrates patients with an increase in DI. Plot B shows patients with low or no dysbiosis and with minor changes in DI after 6 weeks.

CONCLUSION

Both LFD and ND groups reported significant reduction in IBS-SSS from week 0 to 6.
High proportions (87%) of the IBD-IBS patients were dysbiotic at baseline.
At week 6, 56% of the patients showed alterations in microbiota.
Alterations in microbiota was not significant between LFD and ND groups after 6 weeks .

UNITED EUROPEAN GASTROENTEROLOGY ueg week
22nd United European Gastroenterology Week
October 18 – 22, 2014 | Vienna, Austria

Lovisenberg Diakonale Hospital

Influence of a low-FODMAP diet on symptoms and gut microbiota in patients with irritable bowel syndrome

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Introduction

Reducing intake of fermentable oligo-, di- and monosaccharides and polyols (FODMAP) may improve functional bowel symptoms. We aimed to investigate the effect of such a dietary change on intestinal and extra-intestinal symptoms and gut microbiota in patients with irritabel bowel syndrome (IBS).

Methodology

IBS patients admitted to Lovisenberg Diakonale Hospital were investigated consecutively from April 2013 to January 2014. Organic diseases were excluded by appropriate investigations. Specifically, all patients underwent a D-xylose breath test to exclude small intestinal malabsorption. Symptoms were assessed by using validated questionnaires to measure both intestinal (IBS-SSS) and extra-intestinal symptoms (HADS, FIS) before and after 4 weeks on a low-FODMAP diet. Fecal gut bacteria DNA analysis was performed by using the GA-map™ Dysbiosis Test (Genetic Analysis AS, Oslo, Norway; figure 1). This 16S rRNA DNA test utilizes DNA probes to recognize gut bacteria (1) found best to correlate with dysbiosis in patients with IBD and IBS. Dysbiosis index is an index calculated by an algorithm based on bacterial abundance and profile in a fecal sample, measured on a scale from 1 to 10, where values above 2 are considered abnormal. Changes in dysbiosis indices between week 0 and 4 were investigated.

Results

Forty-eight patients (4 M, 44 F) completed the study. At baseline, 23 and 25 patients had a dysbiosis index classified as "normal" and "abnormal", respectively. These two groups were significantly different regarding intestinal symptom severity (mean IBS-SSS scores 263 versus 304, respectively; P = 0.04), but similar regarding extra-intestinal symptom severity. A correlation between dysbiosis index and IBS-SSS was demonstrated (r = 0.29, P = 0.04; figure 2), including the subscale measuring pain (r = 0.30, P = 0.04; figure 2). Following dietary intervention, symptomatic improvement was demonstrated as a reduction in IBS-SSS (from 285 to 157; P < 0.0001), HADS (from 14 to 9; P < 0.0001) and FIS (from 72 to 38; P < 0.0001). The dysbiosis index changed in 31 (65%) patients while it remained unchanged in 17 (35%) patients. There was no correlation between change in dysbiosis index and change in symptoms following diet.

Figure 1
Method for assessing composition of fecal microbiota by using the GA-map™ Dysbiosis Test (Genetic Analysis AS, Oslo, Norway)

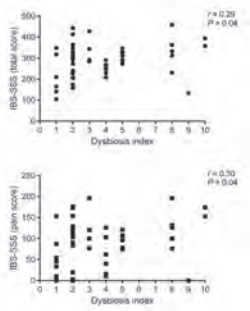

Figure 2
Relationship between dysbiosis index and IBS-SSS at baseline. Upper panel: Total scores. Lower panel: Pain scores.

Conclusion

A low-FODMAP diet seems to improve not only intestinal, but also extra-intestinal symptoms in patients with IBS. The GA-map™ Dysbiosis Test showed that patients with higher dysbiosis indices had more severe intestinal symptoms at baseline. The test thus provides information on alterations in bacterial abundance and profiles that may prove valuable for individual patients. However, we did not demonstrate any associations between change in dysbiosis indices and symptoms following dietary intervention.

Reference
1. Vebø HC et al. Clin Vaccine Immunol 2011; 18: 1326-35.

Conflict of interests
The study was partly supported by a research grant from Genetic Analysis AS

UNITED EUROPEAN GASTROENTEROLOGY ueg week
22nd United European Gastroenterology Week
October 18 – 22, 2014 | Vienna, Austria

Poster P1000
21 October 2014

Gut Microbiota alterations in IBS patients before and after 6 weeks of low FODMAP diet versus *Lactobacillus Rhamnosus* GG

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Introduction

Low FODMAP diet (LFD) and probiotics may be effective in patients with IBS, and these patients may have an altered microbiota.
The aim of the study was to investigate the impact of LFD and *lactobacillus rhamnosus GG* (LGG) on fecal microbiota.

Materials and Methods

- 59 IBS patients (ROME III)
- Intervention groups: LFD, LGG or normal Western/Danish diet (ND)
- Week 0 and 6: Fecal samples collected and IBS-SSS registered (www.ibs.constant-care.dk)
- Microbiota analysis; GA-map™ Dysbiosis Test* (16SrRNA)
  - Utilizing 16SrRNA in bacteria detection
  - Measures the degree to which an individual's gut microbiota differs from that of a healthy reference population
  - Dysbiosis Index score (DI) is calculated by an algorithm based on bacterial abundance and profile in a fecal sample
  - Measured on a scale from 0-5, where values above 2 are considered dysbiotic.

		Gender	Age	
Sample	Number	(% Female)	Mean	Min-Max
LFD	18	89	40.3	22-71
LGG	20	80	41.6	23-74
ND	21	76	38.2	20-73

Table 1. Patients demographics

Results

IBS-SSS

	IBS-SSS week 0		IBS-SSS week 6		
Sample	Mean	Min-Max	Mean	Min-Max	P-value
LFD	310	150-460	193	25-478	0.0003
LGG	296	157-431	212	11-471	0.004
ND	303	82-450	289	62-428	0.55

Table 2. IBS-SSS at baseline and after 6 weeks intervention

Dysbiosis (DI scores)

	Dysbiotic (%)		DI (mean)		DI
Sample	Week 0	Week 6	Week 0	Week 6	P-value
LFD	89	78	3.33	3.33	1
LGG	65	75	3.2	3.35	0.6
ND	76	81	3.2	3.2	0.9

Table 3. DI scores at baseline and after 6 weeks intervention

Change in dysbiosis status

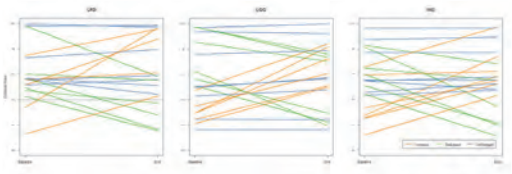

Figure 1. DI at baseline and end intervention

Bacteria observations after intervention

- In patient with abnormally high levels of *Akkermansia muciniphila* (Verucromicrobia); dietary intervention (LFD or LGG) did not seem to normalize gut microbiota or remove dysbiosis.
- LGG treatment in patients with high levels of *Lactobacillus* and *Streptococcus salivarius thermophilus* (Firmicutes (Bacilli)) seemed to either cause dysbiosis (non-dysbiotic becomes dysbiotic) or result in persistent severe dysbiosis.
- The results showed significant reduction in levels of Bacteroides/Prevotella, *Faecalibacterium prausnitzii* and Firmicutes (Lachnospirae) after LFD treatment, while increase in *Eubacterium hallii*.

Conclusions

- Both LFD and LGG groups reported significant decrease in IBS-SSS after 6 weeks of treatment.
- No significant improvement in DI after week 6 weeks
- No correlation between change in IBS-SSS and DI
- High proportions (65-89%) were dysbiotic at week 0
- Alteration in microbiota was observed in 35-43% of the patients who changed dysbiosis class following dietary intervention.
- The test provides information on alterations in bacterial abundance and profiles that may prove valuable for individual patients.

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STABILITY OF REPEATED FECAL DNA PREPARATION USING THE GA-MAP™ DYSBIOSIS TEST

Caroline Jevanord Frøyland[1], Annette Mahler[2], Christina Casén[1], Kari Stenersen[1], Magdalena Kauczynska Karlsson[1]

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Introduction

The composition of the human intestinal microbiota is being increasingly studied as a regulatory factor in health and disease. In recent years, several reports elevate the importance of standardized sample preparation when working with complex bacteria community samples such as feces. DNA extraction has been introduced as one of the main reasons for low reproducibility of microbiota results between labs and/or methods. Bias in downstream applications can be introduced if a non-optimized non-standardized method is being used.

The aim of the study was to verify repeatability and reproducibility of fecal sample DNA preparation using the GA-map™ Dysbiosis Test.

Materials and Methods

Fecal samples from 11 donors were collected: 3 were used for a study of within run repeated DNA extraction, while 8 were used for a study of laboratory-laboratory repeatability and reproducibility. The 8 samples were homogenized and aliquoted before further procedure.

Samples were analyzed using GA-map™ Dysbiosis Test, a novel gut bacteria DNA test utilizing the 16S rRNA gene[1]. To measure the degree to which an individual's gut microbiota differs from that of a healthy reference population, a set of 54 selected probes is being used targeting gut bacteria and bacteria groups important in human health. The test procedure includes a standardized method for fecal DNA extraction comprising, among others, mechanical lysing, protease treatment, and extraction using MagMAX™ express 96 DNA extraction robot. For details see Casén et al. (2015)[1].

The Test generates a Dysbiosis Index score (DI) calculated by an algorithm based on bacterial abundance and profile in a fecal sample compared to a documented healthy reference cohort. The microbiota dysbiosis is measured on a scale from 0-5, where values above 2 are considered dysbiotic. Additionally, abundance of 15 selected bacteria is reported on a level scale from -3 to 3.

Results – repeated extraction within one run

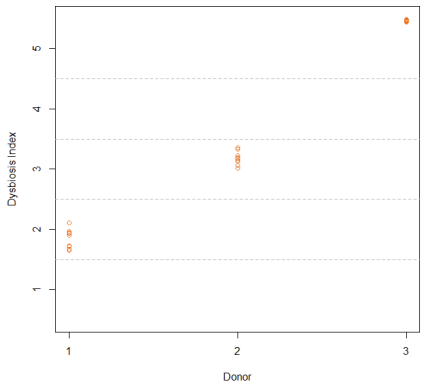

Figure 1. Dysbiosis Indices for 10 repeated DNA extractions from 3 fecal samples.
Three previously analyzed fecal samples (normal, weak dysbiosis and severe dysbiosis) were extracted simultaneously 10 times by one operator. All 10 fecal aliquots per donor showed identical Dysbiosis Indices (DI) with standard deviation ≤0.15.

Results - repeated extraction between laboratories

Fecal samples from 8 donors were processed and analyzed at two laboratories (Norway and Germany). DNA was extracted in duplicate and analyzed in triplicate (n=48) at each laboratory. For laboratory 1, all 48 samples passed QC, while for laboratory 2, 42 samples passed.

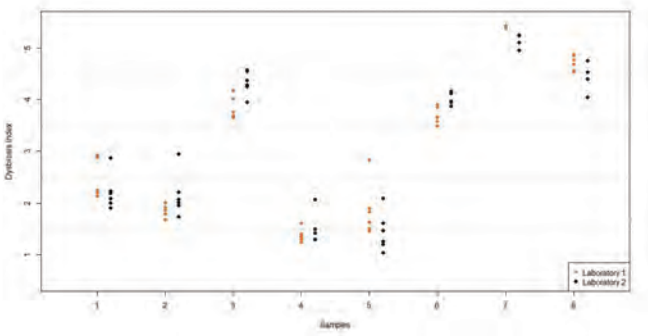

Figure 2. DI for samples processed by two different laboratories.
To compare DI values between laboratories, laboratory 1 (Norwegian, orange) was used as the reference laboratory and a technical standard deviation (SD) of 0.3 was applied. Of 42 DI values from laboratory 2 (German, black), 35 fall within a 2SD limit with a pass rate of 83%

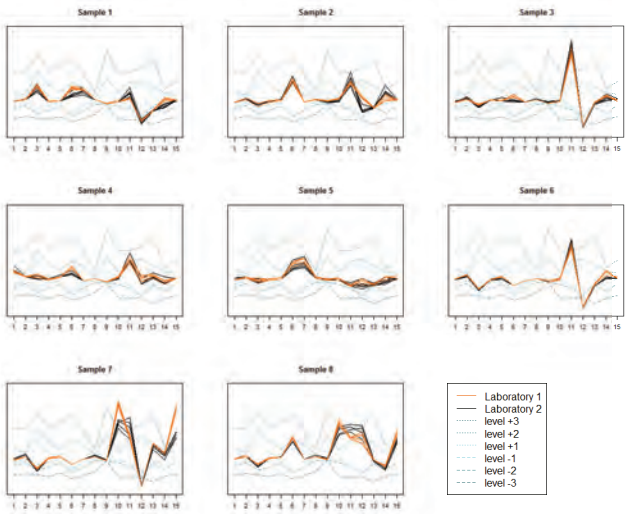

Figure 3. Relative levels of bacteria by repeated DNA extraction and analysis at two laboratories.
Microbiota profiles between laboratory 1 (Norwegian, orange) and laboratory 2 (German, black) were compared. 35 out of 42 profiles (83%) were equivalent and within ±1 level between laboratories. The area between level -1 and +1 is normal range. Numbers from 1 to 15 (x-axis) represent the selected bacteria/bacteria groups: *Ruminococcus albus/bromii (1), Ruminococcus gnavus* (2), *Faecalibacterium prausnitzii (3),* Lactobacillus (4), *Streptococcus sanguinis* and *Streptococcus salivarius* ssp.*thermophilus* (5), *Dialister invisus* (6), *Akkermansia muciniphila* (7), *Bacteroides fragilis* (8), Alistipes (9), Shigella/ Escherichia (10), Bifidobacterium (11), Bacteroides/Prevotella (12), Firmicutes (Bacilli) (13), Firmicutes (Clostridia) (14) Proteobacteria (15).

Conclusions

DNA preparation utilizing the standardized GA-map™ Dysbiosis Test showed:

- Excellent repeatability of multiple DNA extractions of fecal samples with identical Dysbiosis Indices
- Good repeatability and reproducibility *between laboratories* with similar Dysbiosis Indices and bacterial profile
- Using standardized and optimized methods for analysis of fecal microbiota, such as the GA-map™ Dysbiosis Test, ensures precise and stable results.

References

[1] Casén C *et al.* Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. *Aliment. Pharmacol. Ther.* 1–13 (2015). doi:10.1111/apt.13236

UEG Week 2015 - Abstract Submission

Topic area: 7. LOWER GI (EXCLUDING IBD)
(PLEASE SEE SURGERY AND ENDOSCOPY SECTIONS FOR FURTHER OPTIONS)

Topic: 7.14. Irritable bowel syndrome: Treatment

UEG15-ABS-1686

MONITORING GUT MICROBIOTA DURING PROBIOTICS TREATMENT IN A PATIENT SUFFERING FROM LONG TERM FUNCTIONAL GASTROINTESTINAL DISORDER

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[1]Gastromedical, Akershus University Hospital, Lørenskog, [2]Clinical and Regulatory, [3]Production, Genetic Analysis AS, Oslo, Norway

Has this abstract previously been presented?: No
Has this abstract been previously published?: No
Please select "Yes" in case your abstract should be considered as "Translational/Basic Science".: No
This abstract should be taken into consideration for the "Today's science; tomorrow's medicine" sessions.: Yes
Does the presenting author fulfil the criteria and want to apply for the travel grant?: No
Contact E-mail Address: cc@genetic-analysis.com

Introduction: The aim is to evaluate if monitoring of microbiota in a patient suffering from long term functional gastrointestinal disorder is a useful tool in treatment and follow-up of patients.

Aims & Methods: The patient is a 37 year old female with chronic diarrhea the last 10 years after an infection with Salmonella enteritidis. Blood and fecal samples have been negative. Gastroscopy with duodenal biopsies and ileocolonoscopy with biopsies on several occasions without pathological findings. Very rapid bowel transition as only objective finding. Diagnosed with irritable bowel syndrome with diarrhea (IBS-D). Currently on lactose and gluten free diet, as well as low FODMAP diet. Smoking 10 cigarettes/day. Fecal samples were collected at the following time-points; 1) Baseline, before onset of probiotic treatment, 2) After one month of treatment with a half dose of probiotics ($225x10^9$ CFU) (VSL#3, Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD, USA), 3) After one month off probiotic treatment, and 4) After another month treatment with a half dose of probiotics (data not shown). Microbiota analysis was performed with the GA-map™ Dysbiosis Test (1), (Genetic Analysis AS, Oslo, Norway). This 16S rRNA DNA test utilizes DNA probes to recognize gut bacteria found to best correlate with dysbiosis in patients with IBD and IBS.

Results: Changes in the gut microbiota was observed after probiotic treatment. The most pronounced changes were observed for the bacteria phyla Proteobacteria and for the genus Shigella and Escherichia. During the treatment periodthese bacteria were reduced from very high abundance to a normal level. After a short period without treatment the Proteobacteria abundance was increased. In addition, an increase in *Bacteroides fragilis* species was observed only during treatment. Many members of Proteobacteria phyla are known to be opportunistic, while *B. fragilis* is a commensal bacterium (2). Furthermore, bacteria belonging to the family Lachnospiraceae and species *Faecalibacterium cf. prausnitzii* and *Staphylococcus epidermidis* were more abundant in the sample after treatment. Low levels of the commensal bacteria *F. prausnitzii* and Lachnospiraceae are associated with Crohn's Disease (3).

Conclusion: The GA-map™ Dysbiosis Test reveals significant differences in microbiota correlated to probiotic treatment and can be a useful tool in longitudinal follow-up of patient in treatment. Any major clinical response has so far not occurred in the patient examined in this study, and a longer treatment period might be needed to achieve clinical effect. It can, however, be concluded that the test is a useful tool in detecting and monitoring the gut microbiota and that microbiota measurement is a useful tool in longitudinal follow-up of patients in treatment.

References: 1. Casen C et al. Deviations in human gut microbiota: A novel diagnostic test for determining dysbiosis in IBS and IBD patients. Aliment Pharmacol Ther 2015 (in press).

2. Mazmanian SK, et al. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008. 453(7195):620-5

3. Fujimoto T, et al. Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease . J Gastroenterol Hepatol 2013; 28(4):613-9

I confirm having declared any potential Conflict of Interest for ALL authors listed on this abstract: Yes

Disclosure of Interest: None Declared

Keywords: Microbiota, probiotics

European Crohn's and Colitis Organisation

A novel diagnostic test for determining microbial dysbiosis

by Genetic Analysis AS, a partner in the IBD Character consortium

'Inflammatory Bowel Disease Characterization by a multi-modal integrated biomarker study'

Amsterdam, March 16th 2016

Christina Casén, GA

Genetic Analysis

IBD Character

IBD Character

- Collaborative effort to advance our understanding of IBD
- Increase diagnostic precision in detection of the diseases in their early manifestation
- Treatment naïve patients recently diagnosed with inflammatory bowel disease
- In order to create a molecular snapshot of IBD in its early manifestation, the project will analyse:
  - DNA methylation status
  - RNA transcription profile
  - Protein markers
  - Gut microbial content in mucosa and feces

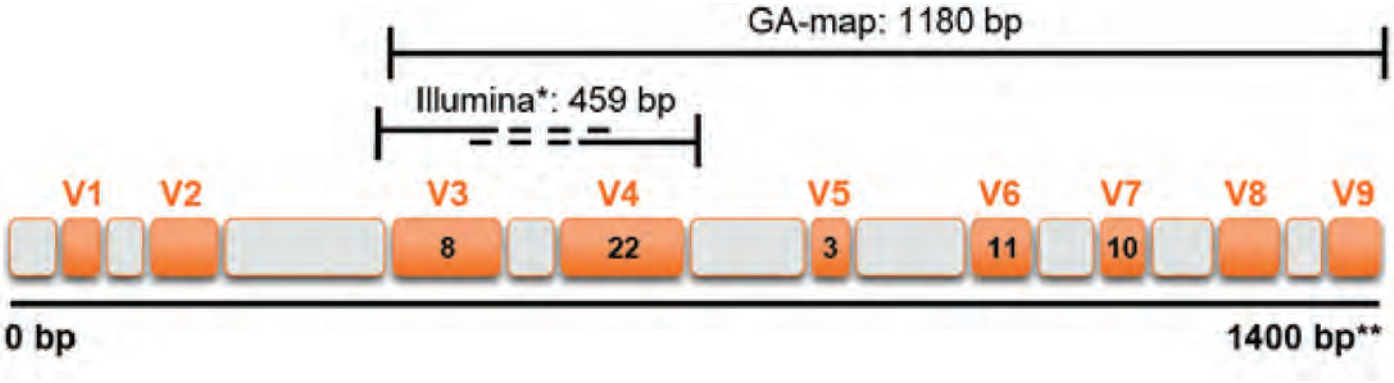
The patient cohort

Gut microbiota profiles in treatment naïve

- IBD
- non-IBD patients
- and in healthy controls

GA-map™ Dysbiosis Test

Bacterial 16S rRNA gene



Pre-determined bacteria

- 300 bacteria at different taxonomic level
- Based on 54 DNA probes in recognizing gut bacteria profiles

***Casén et al. Aliment Pharmacol Ther 2015; 42: 71–83.*

IBD Character

GA-map™ Dysbiosis Test*

Definition of Dysbiosis

Dysbiosis = imbalance in the gut microbial community

GA-map™ Dysbiosis Index (DI):

- DI score of 1-2 is defined as non-dysbiotic and
- DI score from 3-5 is defined as dysbiotic

*Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. *Casén et al. Aliment Pharmacol Ther 2015; 42: 71–83.*

IBD Character

IBD Character Cohort overview

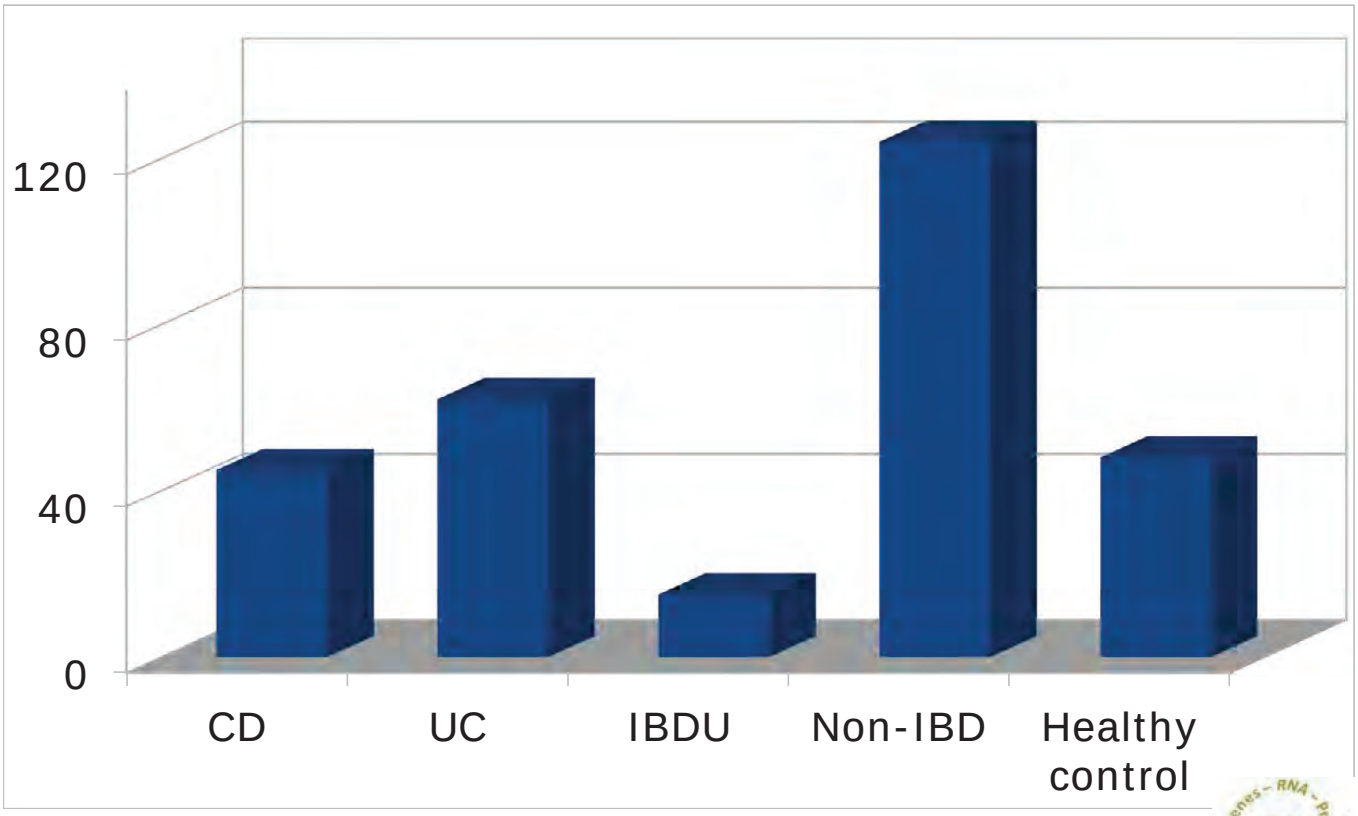
Adult population; fecal samples analysed for microbiota profiles	**N= 345**
Eligible adult population (53* subjects left out due to incomplete clinical record)	**N= 292**

* The data will be re-analyzed when clinical records are completed for all subjects

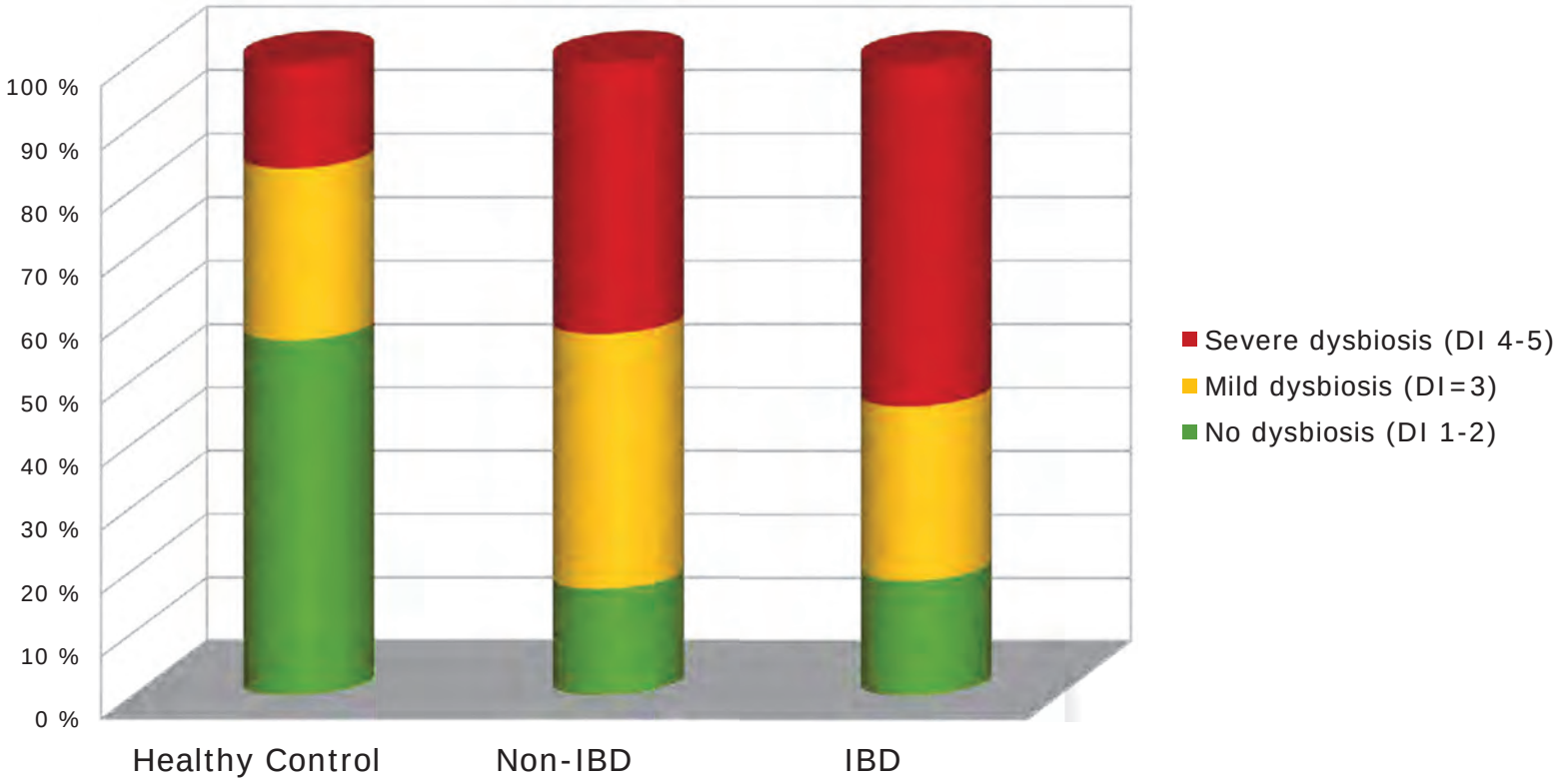
Patient recruitment overview

Partner	Subjects (#)
Oslo, Norway	92
Ørebro, Sweden	71
Linköping, Sweden	31
Edinburgh, UK	43
Zaragosa, Spain	55
TOTAL	292

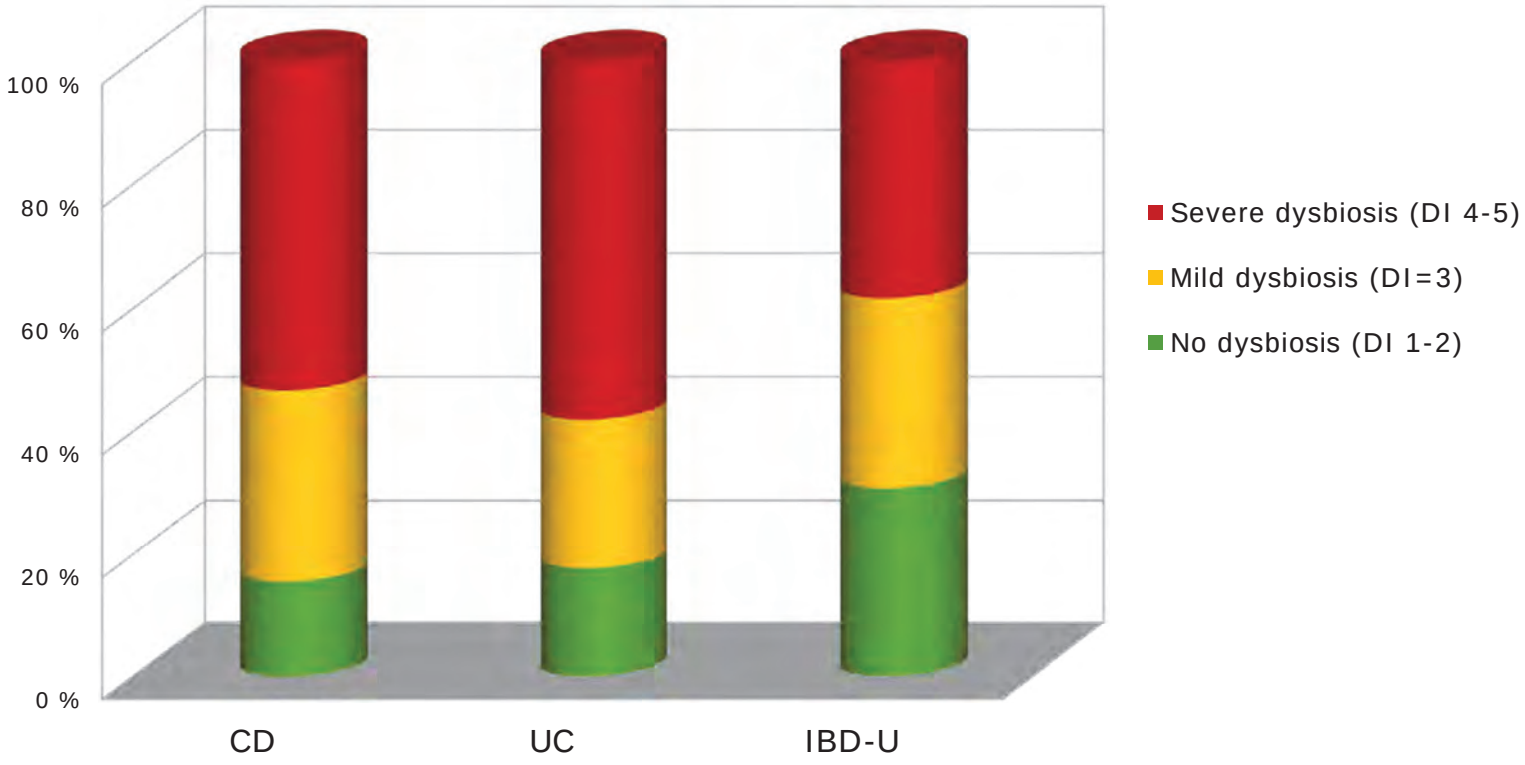
Distribution of patients in diagnose group N=292



Distribution of dysbiosis within the diagnose groups N=292



Distribution of dysbiosis within the IBD subgroups N=120

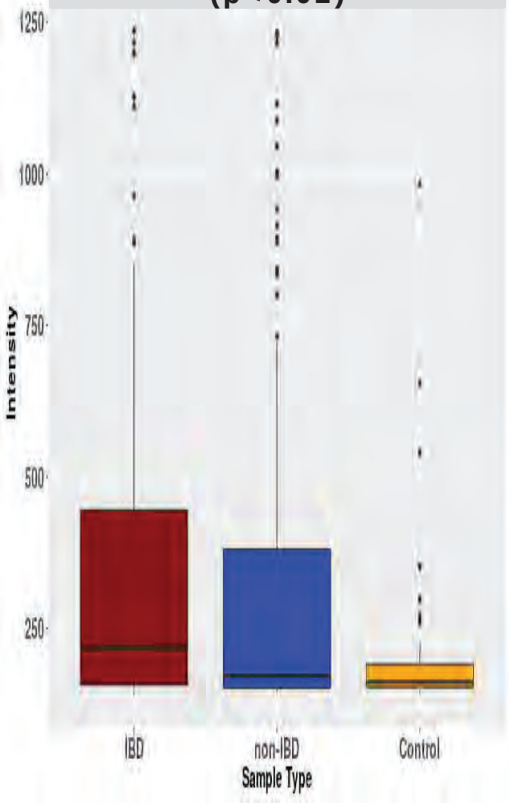


IBD Character

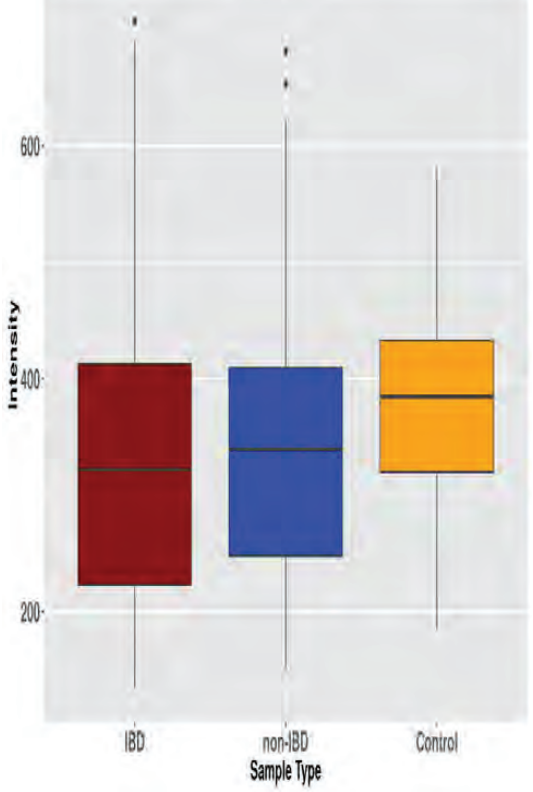
Difference in bacteria profiles

IBD vs. non-IBD vs. healthy control

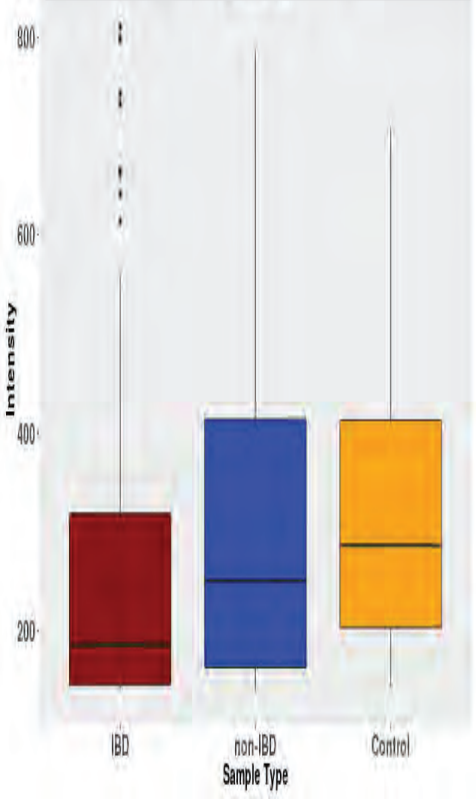
Escherichia (Proteobacteria) (p<0.02)



Faecalibacterium Prausnitzii (p<0.07)



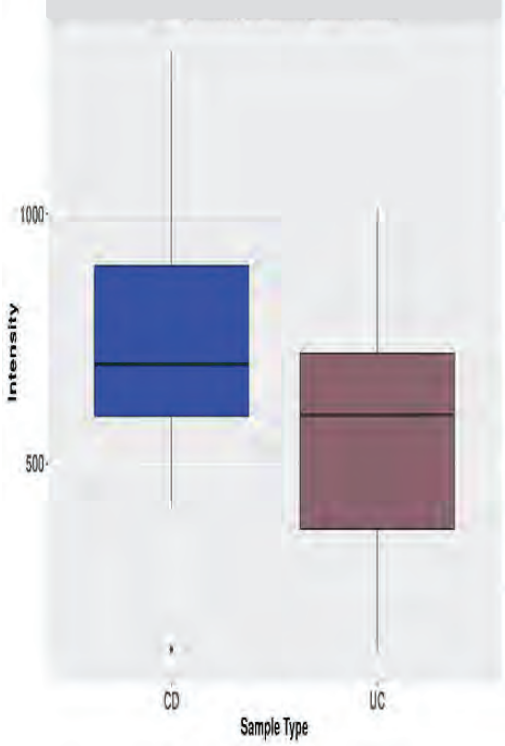
Bifidobacterium (p<0.02)



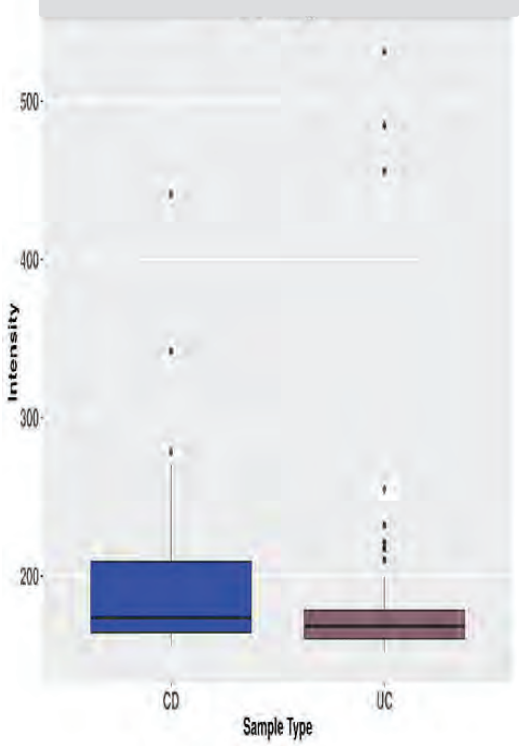
Difference in bacteria profiles

CD vs. UC ($p<0.05$)

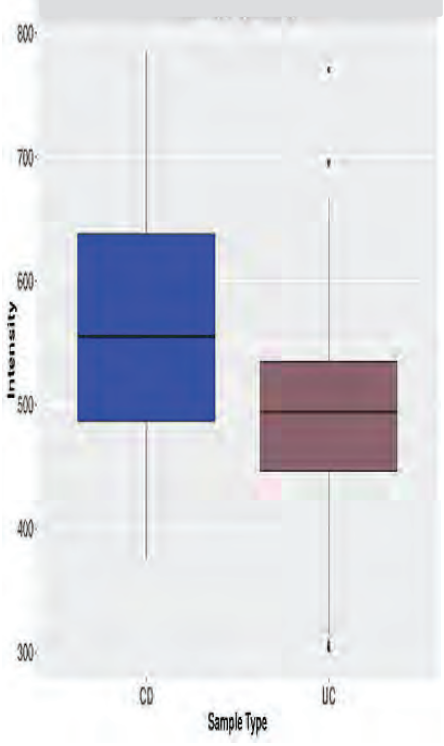
Firmicutes



Streptococcus

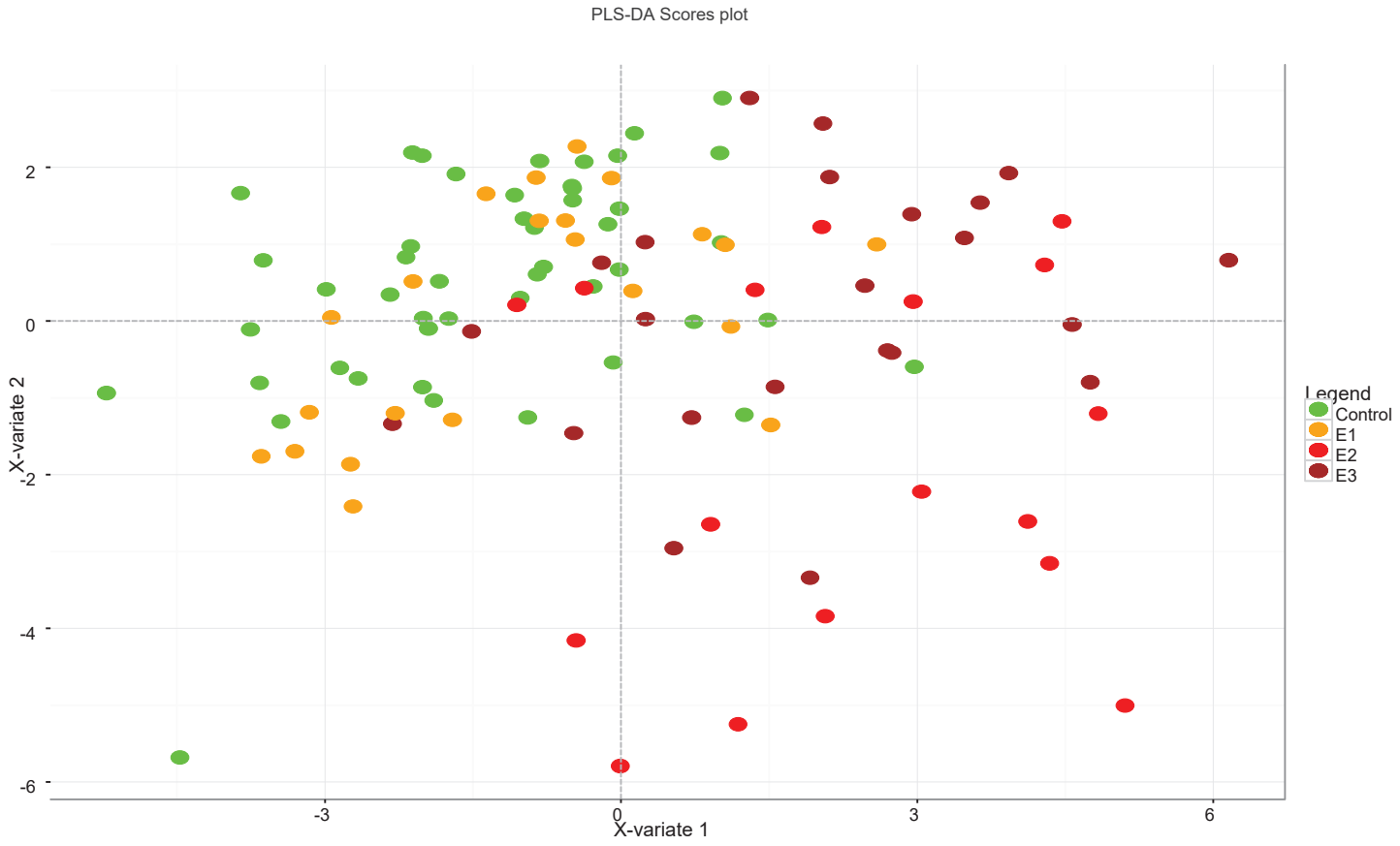


Clostridia



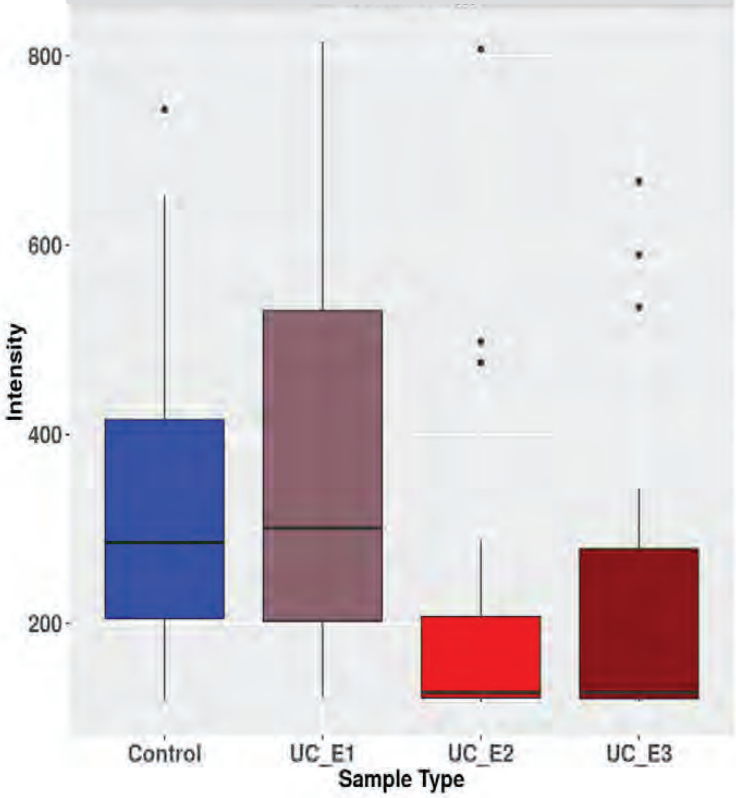
Results

UC proctitis (E1) vs. leftsided/pancolitis (E2/E3) vs. control N=108

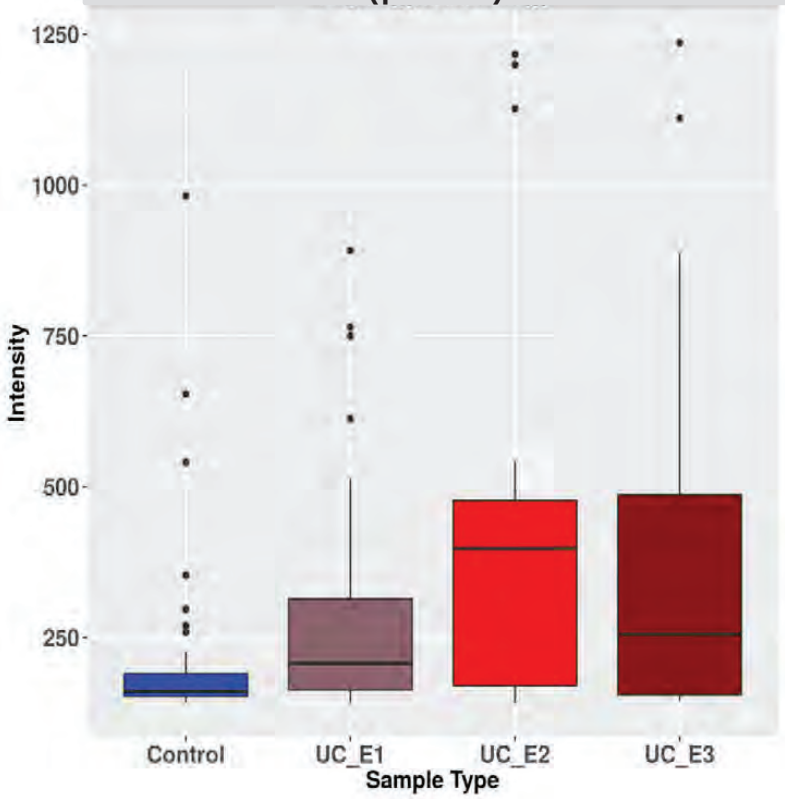


Difference in bacteria profiles
UC subgroups E1, E2, E3 and healthy controls

Bifidobacterium
(p<0.01)



Escherichia (Proteobacteria)
(p<0.01)



Summary

- Developing a diagnostic test for determining microbial dysbiosis is accomplished
- The test is a tool to measure alterations in microbiota profiles

In conclusion

- The results demonstrate differences in microbiota profiles between
  - IBD, non-IBD and healthy controls
  - CD and UC
  - Subgroups of UC
- The results support that alterations in microbial composition is important both in IBD and non-IBD

Abstract 2801

MICROBIOTA ALTERATIONS IN TREATMENT NAÏVE IBD AND NON-IBD PATIENTS - THE EU IBD-CHARACTER PROJECT

Type: Abstract

Topic: 05. IBD (INCLUDING MICROSCOPIC COLITIS) / 5.04. Diagnosis and monitoring

Authors: S. Vatn[1], P. Ricanek[1], R. Kalla[2], Y. Ber[3], E. Ciemniejewska[1], M.J. Pierik[4], J. Halfvarson[5], J.D. Söderholm[6], J. Jahnsen[1], F. Gomollon[3], J. Satsangi[2], M.H. Vatn[7], M. Sekelja[1], C. Casén[1], &. the IBD-Character consortium[1]; [1]Oslo/NO, [2]Edinburgh/GB, [3]Zaragoza/ES, [4]Maastricht/NL, [5]Örebro/SE, [6]Linköping/SE, [7]Lørenskog/NO

Introduction

The microbiota is considered important for development of intestinal diseases. In order to create a molecular snapshot of IBD in its early manifestation, one part of the IBD-Character project identified faecal microbiota profiles among the strictly treatment naïve IBD and symptomatic non-IBD patients, and a healthy control group.

Aims & Methods

Patients where characterized by international criteria including endoscopy and biopsies. Faecal samples collected during five days prior to diagnosis where stored at – 80°C before examination on GA-map™ Dysbiosis Test (1), a 16S rRNA DNA test utilizing DNA probes to recognize gut bacteria profiles. In total 54 probes have been selected (1) for recognition of dysbiosis in IBD/non-IBD patients and normal controls.

Results

Table 1. Dysbiosis status

Dysbiosis	Patients	Age [med.]	Female	IBD	CD	UC	IBDU	Non-IBD	Healthy control
No	72	28 (19-68)	43	22 [18%]	7 [16%]	11 [18%]	2 [100%]	21 [17%]	27 [56%]
Low	96	33 (19-66)	49	33 [28%]	14 [31%]	15 [24%]	0	50 [40%]	13 [27%]
High	126	32 (18-69)	80	65 [54%]	24 [53%]	36 [58%]	0	53 [43%]	8 [17%]
Total	294	NA	172	120	45	62	2	124	48

In total 294 adult patients and control subjects were investigated for microbiota profiling. Table 1 shows the distribution and frequency of dysbiosis in the diagnose groups, subgroups and healthy controls.

Most IBD patients were in the high dysbiosis group (54%), while for non-IBD patients high and low dysbiosis was equally distributed (40 and 43%, respectively). In the healthy control group most subjects were in the non- and low-dysbiosis groups (56 and 27%, respectively). Comparing the bacteria profiles of IBD, non-IBD and control groups, the abundance of Proteobacteria was increased in IBD and non-IBD as compared to the controls ($p<0.02$), while the abundance of Bifidobacterium and Faecalibacterium prausnitzii was decreased ($p<0.02$ and <0.07, respectively). Concerning the CD and UC subgroups, a significantly reduced abundance of Firmicutes, Streptococcus and Clostridia was found in UC patients ($p<0.05$). Looking at the microbiota profiles of the Montreal classified subgroups of the UC patients, as compared to the healthy controls in a PLS analysis, the healthy controls (n=48) and E1 (n=22) patients clustered together, while the combined group of E2 (n=17) and E3 (n=23) patients made a separate cluster. Looking at two of the bacteria groups in details, Bifidobacterium were significantly reduced ($p<0.01$) and Escherichia/Proteobacteria were significantly increased ($p<0.01$) in the E2/E3 group as compared to E1/ healthy controls group.

Conclusion

The results demonstrate differences in microbiota profiles between IBD, non-IBD and healthy controls, CD and UC and subgroups of UC. Moreover, IBD in general seems more dysbiotic than non-IBD. The frequency of high dysbiosis among the control subjects (17%) was higher than observed in other studies (1). Apart from showing increased frequency and degree of dysbiosis for both UC and CD, UC showed significantly lower abundance than CD patients in some specific microbial genera. In UC, the alteration in bacterial abundance in E2/E3 as compared to E1/healthy controls, may indicate that the microbiota profiles are dominated by different bacteria dependent upon the localization of the inflammation in these patients. The present results support that alterations in microbial composition is important in both IBD and non-IBD.

References

(1) Casén et al. Aliment Pharmacol Ther 2015; 42: 71–83

Low FODMAP diet alters symptoms, microbiota, short-chain fatty acids and cytokine profiles in patients with IBS: A randomized controlled trial

Hustoft TN[1], Hausken T[1,2,3], Ystad SO[1,3], Valeur J[4], Brokstad KA[5], Hatlebakk JG[1,2,3], Lied GA[1,2,3]

[1]Department of Clinical Medicine, University of Bergen, [2]Section of Gastroenterology, Department of Medicine, Haukeland University Hospital Bergen, [3]National Centre of Functional Gastrointestinal Disorders, Haukeland University Hospital Bergen, [4] Unger-Vetlesen`s Institute, Lovisenberg Diakonale Hospital, Oslo, [5]Department of Clinical Science, University of Bergen, Norway

Objective: To investigate the effect of a low versus high FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diet on symptoms, gut microbiota, short-chain fatty acids (SCFAs) and pro-inflammatory cytokine profiles in a randomized, double-blinded, crossover trial of Norwegian patients with irritable bowel syndrome (IBS).

Materials and methods: Twenty patients with IBS (15 female/5 male, mean age 34.6 y) were instructed to follow a low FODMAP diet (LFD) throughout a study period of 9 weeks. After 3 weeks they were randomized and double-blindly assigned to receive a daily supplement of either high (16 g fructo-oligosaccharides (FOS)) or low (16 g maltodextrin (= placebo)) FODMAP for the next 10 days, followed by a 3-week washout before crossing-over to the alternative supplementation for 10 new days. IBS Severity Scoring System (IBS-SSS) was used to evaluate symptoms. Blood samples were collected to analyse serum cytokines (IL-6, IL-8, TNF-α), and faeces samples for gut microbiota (s16r RNA) and SCFAs.

Results: IBS symptoms consistently and significantly improved after 3 weeks of LFD, with a mean overall reduction of 163.8 points ($p < 0.0001$). On average, 4 of 5 symptoms were significantly worsened in response to FOS compared with placebo, with an overall difference of 65.1 points ($p = 0.014$). Serum levels of IL-6 and IL-8, but not TNF-α, significantly decreased on the LFD ($p = 0.001$ and $p < 0.0001$, respectively). The same did apply to luminal *Faecalibacterium prausnitzii* and *Bifidobacterium* ($p = 0.0084$ and $p = 0.0094$, respectively). Levels of total SCFAs and butyric acid were also significantly decreased on the LFD ($p = 0.04$ and $p = 0.01$, respectively). Ten days of FOS supplementation normalized the level of bacteria, but did not change the levels of cytokines nor SCFAs.

Conclusion: FODMAP content was related to IBS symptoms, cytokine levels and selected intestinal bacteria species. Our results provide evidence to support the efficacy of a LFD in reducing functional GI symptoms. Further studies are warranted to explore the link between FODMAPs, gut microbiota and immune activation.

HARRIET GUTHERTZ
MEDICAL MARKETING AND COMMUNICATIONS
UEG ABSTRACT – GA MAPPING
APRIL 26, 2016

Consistent and Reproducible Production of a Microbiota-based Drug for Recurrent C. difficile Infection: Application of a Novel Diagnostic for Dysbiosis

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Background: Antibiotics have been the first-line treatment for C. difficile infection (CDI). However, the most commonly prescribed antibiotics for CDI are associated with high recurrence rates. Antibiotics have been shown to disrupt the intestinal microbiota. Restoration of the intestinal microbiota to its pre-disease state protects against recurrence. There is an unmet need for a standardized, reproducible microbiota-based therapy for recurrent CDI.

RBX2600, a microbiota-based drug candidate targeted at recurrent CDI, is sourced from human-derived microbes from extensively screen donors and manufactured using standardized, quality controlled processes.

Objective: To compare bacteria abundance in the RBX2660 source material (DS) with the processed drug product (DP) used in the Phase 2B PUNCH CD 2 study.

Methods: A total of 70 DS samples sourced from 17 donors (mean age 27; range 18 to 57 years; 94% male) between August 2014 to February 2016 were compared with 70 matched DP samples using the GA-map Dysbiosis Test (GA), Genetic Analysis AS, Oslo, Norway. The GA-map uses 54 probes targeting V3 to V7 of the bacterial 16s rRNA gene to characterize and identify bacteria present in order to determine if a person's microbiome displays health or dysbiosis. The GA diagnostic covers approximately 300-400 bacteria at different taxonomic levels and provides an assessment of the microbial community by using multiple variable regions. The GA diagnostic enables serial assessment of fecal bacterial community abundance profile and potentially clinically relevant alterations in the microbiome over time.

Results: GA analysis found that bacterial abundance in the DP was lower in number than in the DS in 38 of the 54 probes; equal in number in 6 of the probes; and higher in 10. More specifically, *Firmicutes* and *Actinobacterium* showed reduced signals in the DP compared with the DS. *Bacteroidetes* showed increased signal strength in the DP compared with the DS, while *Proteobacteria* demonstrated equal signal in both samples. Accuracy was as high as 83.4% at cross-validation. Principal component analysis found that bacteria profiles in RBX2660 drug product, though lower than in the donor source material, were largely kept intact during the production process for all 17 donors. This demonstrates that the diversity of a healthy microbiome, believed to be critical for protection from recurrent CDI, is conserved in the DP delivered to a patient in the form of RBX2660.

Conclusions: The GA analysis confirmed that RBX2660 can be manufactured in consistent and reliable manner with preservation of key bacterial diversity.

Reference:

1. Kelly CP, Lamont JT. *Clostridium difficile* — more difficult than ever. *N Engl J.Med.* 2008;359:1932–40.

2. Casén C, Vebø HC, Sekelja M, et al. Deviations human gut microbiota: A novel diagnostic test for determining dysbiosis in patients with IBS or IBD*. Aliment Pharmacol Ther.* 2015;42:71-83.

Table 1. Comparative Signal Strength of Bacteria

Bacteria	**Signal Strength in DP vs. DS**	**Mean difference (95% CIM)**
Bacteroidetes		
Bacteroides fragilis	Increased	0.07 (0.03, 0.11)
Parabacteroides	Increased	0.12 (0.07, 0.17)
Alistipes	Increased	0.17 (0.11, 0.23)
Firmicutes		
Lachnospirae	Decreased	-0.13 (-0.15, -0.11)
Streptococcus	Decreased	-0,16 (-0,20, -0,13
Negativicutes	Increased	0.03 (0.01, 0.06)
Clostridia	Decreased	-0.18 (-0.20, -0.16)
Actinobacteria		
Bifidobacterium	Decreased	-0.33 (-0.38, -0.28)

DP = drug product; DS = drug source, CIM = confidence interval of mean

Abstract UEGW 2016

Multivariate modelling of gut microbial profiles predicts responsiveness to a diet low in FODMAPs

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*University of Gothenburg, Sweden, ‡Karolinska Institutet, Stockholm, Sweden † Sabbatsbergs Hospital, Stockholm, Sweden

Introduction: Dietary interventions may be recommended to IBS patients yet effects on gut microbiota and factors predicting response are largely unknown.

Aims & Methods: We aimed to determine how two different diets affect gut microbiota and if bacterial profiles and modelling thereof can be used to predict patient intervention response in a secondary analysis of a previously published intervention study (Böhn et.al 2015). After a 10 day screening period 61 IBS patients with at least moderately severe IBS symptoms according to IBS Symptom Severity Score (IBS-SSS) followed either a traditional IBS (n=30) or low-FODMAP (n=31) diet for 4 weeks. Faecal samples were collected and IBS-SSS were completed before and after the intervention. Food intake was recorded in 4-days food diaries before (baseline) and during the interventions. Responders were defined as having a reduction of IBS-SSS ≥50 after the intervention. Faecal bacterial composition was evaluated by GA-map™ Dysbiosis Test which measures probe signal intensity (PSI) of 54 DNA probes targeting ≥300 bacteria on different taxonomic levels. Bacterial profiles created for each patient were evaluated by multivariate discrimination analysis and graded from 1-5, relative to a healthy reference group. A dysbiosis index (DI) ≤2 signify normal microbiota composition and ≥3 signify altered microbiota composition (dysbiosis). For all models, both strong and moderate outliers were sequentially excluded.

Results At baseline, 45 patients (25 randomized to traditional diet and 20 to low-FODMAP) had a DI ≥3, i.e. dysbiosis; of these, 10 patients following the traditional IBS diet and 3 following the low-FODMAP diet experienced an improvement in DI, while 6 following the traditional diet and 11 on the low-FODMAP diet had worsening of their dysbiosis; the rest experienced no change. In the low-FODMAP group, but not traditional diet group, non-responders (n=19) had more severe dysbioisis than responders (n=12) ((3 (3-4) DI; 2 (2-3) DI; *p*=0.007) at baseline. Although patients on a traditional diet consumed significantly less protein, fat and alcohol, they experienced no change in overall bacterial composition after the intervention. Patients on a low-FODMAP diet ate significantly less carbohydrates, fibre, monosaccharides, fructose and total FODMAPS, and had significant reduction in potentially beneficial *Bifidobacterium* after the intervention (33 (25.4-122.4) PSI) compared to before (152 (45.7-70) PSI, *p*=0.0005) which was even more prominent in non-responders. An OPLS-DA model of before the low-FODMAP intervention demonstrated satisfactory modelling and predictive abilities (R2Ycum 0.652, Q2 cum 0.541), showing that bacterial profiles differed between responders and non-responders. An OPLS-DA model of the traditional diet group was inadequate, showing good model fit but poor predictability (R2Ycum 0.742, Q2 cum 0.004), demonstrating that bacterial profiles did not differ between responders and non-responders.

Conclusion Faecal bacterial profiles predict patient responsiveness to a low-FODMAP dietary intervention. Thus, before considering dietary interventions, bacterial profiles could be determined in order to identify patients whom are likely to respond favourably.

Kinetics of microbial community composition in patients with diarrhea-predominant irritable bowel syndrome following faecal microbiota transplantation

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[2]National Centre for Functional Gastrointestinal Disorders, Section of Gastroenterology, Haukeland University Hospital, Helse-Bergen, Bergen, Norway

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Abstract

Introduction
Alterations in gut microbiota are suggested to play an important role in the development of irritable bowel syndrome (IBS) [1]. Through manipulating the gut microbiome of the new host, faecal microbiota transplantation (FMT) has been used to treat patients with treatment-resistant, antibiotic-associated Clostridium difficile colitis [2].

Aim
The aim was to investigate the effect of FMT on the symptoms and on restoring the gut microbiota in patients with IBS.

Methods
The study included 13 patients (4 females and 9 males, age range 20-44 years) with diarrhea-predominant IBS (IBS-D) according to Rome III criteria and 13 healthy asymptomatic donors. The patients received freshly donated feces from a relative and was administered in to the descending part of the duodenum via a gastroscope. Faeces were collected from the donors and the patients before FMT and again from the patients after 1 week and 3 weeks. All the samples were stored in special freezers (-80°C) until analysis. Microbiota analysis was performed using the GA-map Dysbiosis test (Genetic Analysis AS, Oslo, Norway) by algorithmically assessing faecal bacterial abundance and profile (dysbiosis index, DI), and potential deviation in the microbiome from normobiosis [3]. DI is based on 54 DNA probes targeting more than 300 bacterial strains based on their 16S rRNA sequence in seven variable regions (V3–V9). A DI above 2 shows a microbiota profile that differs from that of the normobiotic reference collection (DI 1-2: non-dysbiosis; 3: mild dysbiosis; 4: moderate dysbiosis and 5: severe

dysbiosis) [3]. In addition, the donors and patients completed the following questionnaires before FMT and again for the patients at 3 weeks after FMT to assess the changes in their symptoms and quality of life: IBS symptom questionnaire (IBS-SQ), IBS-symptom severity scoring system (IBS-SSS) and short form of Nepean Dyspepsia Index (SF-NDI).

Results

The DI (mean±SEM) of the donors (1.8±0.23) differed significantly from the patients before FMT (2.7±0.37, P=0.009) and at 1 week after FMT (2.7±0.38, P=0.039) but not at 3 weeks after FMT (2.3±0.29, P=0.1). The profile of a selection of the most important bacteria is presented in table 1.

The scores of IBS-SQ were significantly reduced during the 3 weeks after receiving FMT; total (P<0.0001), nausea (P=0.001), bloating (P<0.0001), abdominal pain (P=0.0005), constipation (P=0.01), diarrhea (P<0.0001), but not for anorexia (P=0.09). The total scores of IBS-SSS, SF-NDI and Bristol stool scale were also significantly reduced after receiving FMT (P=0.0004, 0.004 and 0.008, respectively). No adverse effects were reported after FMT.

Table 1. Mean normalized signal for probes sorted by significant difference between donors and IBS patients before FMT and 1 and 3 weeks after FMT.

Bacteria strain	**Donors**	**Patients**			**P***	**P****	**P*****
		Before FMT	**After 1 week**	**After 3 weeks**	**Before FMT**	**After 1 week**	**After 3 weeks**
Firmicutes, Tenericutes, Bacteroidetes	244±29	128±29	143±32	143±32	**0.014**	0.052	0.31
Ruminococcus gnavus	4.6±1.1	116±68	29±16	26±18	**0.003**	0.097	0.32
Dialister invisus	109±33	15±9.9	47±20	61±37	**0.014**	0.2	0.35
Clostridia, Veillonella, Helicobacter	328±34	227±31	272±36	289±38	**0.025**	0.32	0.5
Lactobacillus, Pediococcus	13±10	3.5±0.2	7.2±3	2.7±0.06	**0.02**	0.07	0.35
Streptococcus	49.3±9.7	79±15.4	48.8±9.6	52±11.3	**0.036**	0.72	0.36
Streptococcus sanguinis and thermophilus	12.2±4.7	67±30.7	26.2±17	29.9±18	**0.007**	0.53	0.43
Anaerotruncus	61.5±0.5	63.2±0.5	62.7±0.3	61.5±0.5	**0.043**	**0.045**	0.98
Bacteroides	144±4.5	169±8.2	149±7.3	143±6	**0.003**	0.79	0.82
Bacteroides, Prevotella	6.7±0.01	6.6±0.02	6.6±0.01	6.7±0.02	**0.04**	0.24	0.59
Proteobacteria	12.4±1.6	26±6	221±91	16.5±3	**0.04**	**0.004**	0.43
Pseudomonas	6.98±0.3	7.99±0.3	7.6±0.2	7.3±0.2	**0.017**	**0.03**	0.14
Shigella, Escherichia	22±6.8	46±16	240±63	40±13	0.095	**0.0003**	0.1
Actinobacteria	159±36	25±4.8	47±10	111±33	**0.0006**	**0.0095**	0.35
Atopobium	4.8±0.1	4.49±0.1	4.47±0.1	4.59±0.1	**0.12**	**0.02**	0.08
Bifidobacterium	189±43	25±5.3	49±11	123±38	**0.0004**	**0.008**	0.28
Actinomycetales	11.4±1.0	8.7±0.9	11.2±1.9	11.1±2.1	**0.03**	0.32	0.42

Data are presented as the mean±SEM. Comparison: Mann-Whitney U test. *Donors vs. patients before FMT, **Donors vs. patients 1 week after FMT, *** Donors vs. patients 3 weeks after FMT.

Discussion

This is the first study to show the kinetics of microbial community composition in IBS patients following FMT. The results of the current study show that FMT helps in restoring alterations in the signals of several strains of the gut microbiota in IBS patients and may have contributed in improving the symptoms and quality of life of the patients.

References

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3. Casen C, Vebo HC, Sekelja M, Hegge FT, Karlsson MK, Ciemniejewska E, Dzankovic S, Froyland C, Nestestog R, Engstrand L, et al: **Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD.** *Aliment Pharmacol Ther* 2015, **42:**71-83.

UNITED EUROPEAN GASTROENTEROLOGY ueg week 24th United European Gastroenterology Week October 15-19, 2016 | Vienna, Austria

P0393

DYSBIOSIS in PATIENTS with FGID

Performance evaluation of dysbiosis status as a tool for clinical investigation in patients with functional gastrointestinal disorders

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Aim of study

- Investigation of microbiota status in patients with functional gastrointestinal disorders (FGID))
- Identification of dysbiosis in the study cohort
- Characterization of any typical microbiota profiles in subgroups of the study cohort

Methods

- In total 99 FGID patients included from 7 private gastroenterology praxes in Germany
- GA-map™ Dysbiosis Test[1] , commercially available test for routine clinical testing
- Utilizing 16S rRNA for bacteria determination
- Dysbiosis Index (DI) score from 1 to 5 (1-2 non dysbiotic, 3-5 dysbiotic)
- Microbiota profile characterization in subgroups of FGID

Results

- Age range 16 to 84 years (median 44 years)
- Sex ratio was 70% females
- Dysbiosis frequency was 69%

Subgroups with different microbiota profiles:

- Low-grade inflammatory patients (N=13) with severe dysbiosis associated with increased *Ruminococcus gnavus* and Proteobacteria, and reduced *Faecalibacterium prausnitzii*
- Post infectious IBS (82% dysbiosis, 9/11 patients)
- Diarrhea predominant FGID (69%, 42/61 patients)

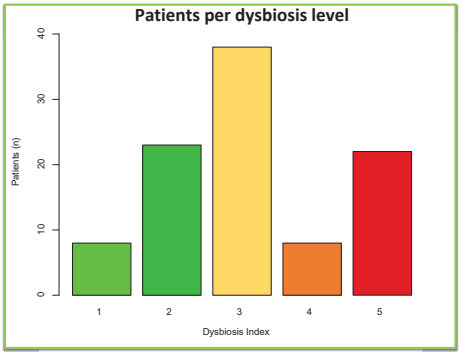

Figure 1. Bar chart of DI distribution. Green-non-dysbiotic; yellow-mild dysbiotic, orange and red-severe dysbiotic.

Bacteria probes	Unadjusted p-value
Post-infectious IBS	
Clostridium	0.0001
Proteobacteria	0.0023
Ruminococcus albus/bromii	0.0034
Akkermansia muciniphila	0.0082
Shigella/Escherichia	0.0117
Streptococcus sanguinis /thermophilus	0.0128
Diarrhea predominant FGID	
Lactobacillus	0.0199
Ruminococcus gnavus	0.0248
Shigella / Escherichia	0.0919
Firmicutes	0.1022

Table 1. Bacteria profiles that most significantly contributed to dysbiosis.

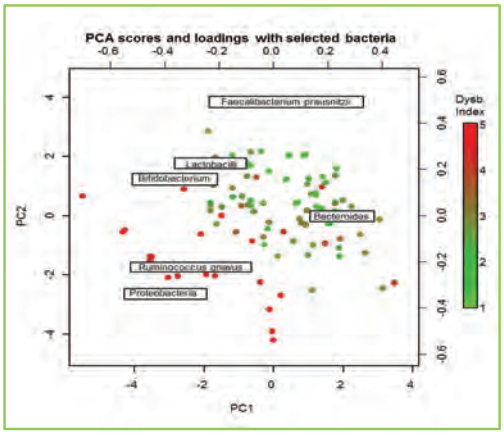

Figure 2. PCA score plot from the first two principal components of the study cohort (n=99 patients) based on 54 DNA probes. The scores are calculated from log-transformed and scaled signal data. Each colored dot represents one patient. The non-dysbiotic patients are in green, moving to dark red for the most dysbiotic patients. These two principal components capture in total 34% of the variation.

Conclusions

- Knowledge about the microbiota status in FGID patients is feasable under clinical routine conditions
- Patient sub-groups can be identified based on similarities in gut bacteria profile
- This tool may reveal 'new' sub-groups in well known diseases like IBS and FGID
- This first routinely applicable dysbiosis test may make targeted therapy possible in FGID patients

1 Casén C et al Aliment Pharmacol Ther 2015;42:71-83

UNITED EUROPEAN GASTROENTEROLOGY ueg week
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Dysbiosis and Stability over two years in Patients with Irritable Bowel Syndrome

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Introduction

- There is increasing knowledge of a possible role for gut microbiota in the pathophysiology of at least subgroups of irritable bowel syndrome (IBS) patients.
- Fluctuations in IBS activity should be reflected by changes in gut microbiota and categorization into a status of dysbiosis or no dysbiosis if there is a causal relationship.
- Dysbiosis status was studied at baseline and after two years.

Aims & Method

- Sixty-three patients with IBS according to Rome III criteria received education about treatment options for IBS by a gastroenterologist and were tested for dysbiosis using the GA-mapTM Dysbiosis Test.
- This is a semi-quantitative 16SrRNA-based analysis of fecal bacteriae (Genetic Analysis, Oslo, Norway). It was repeated two years later.
- Dysbiosis index, DI (1-5) is calculated by an algorithm based on the abundance and profile of bacteria.

Aims & Method (cont.)

- DI 3 or higher is defined as dysbiosis. The abundance of bacteria was measured as low, normal or high.
- We compared the bacterial profile in patients with dysbiosis, those without dysbiosis and any changes in dysbiosis status after two years.

Results 1

- Out of 63 patients at baseline, 60 also provided stool samples after two years.
- 10 (17%) were negative both times (never had dysbiosis = NHD)
  33 (55%) were positive both times (DBT)
  8 (13%) went from no dysbiosis to having it
  9 (15%) went from dysbiosis to losing it.

Results 2

- With focus on the first two groups: abundance of Faecalibacterium prausnitzii, Shigella/Escherichia and Bifidobacterium was significantly lower (Fisher's exact test 0.07, 0.02, 0.04) in the DBT group than the NHD group at baseline, while abundance of Dialister and Bacteroides was significantly higher in the DBT group (0.04, 0.009) after 2 years (see table).

Results 3

In the DBT group:

- the abundance of Ruminococcus gnavus, Lactobacillus, Strectococcus sanguinis and Alistipes species showed high agreement between visits 1 and 2 (82-85%)
- low agreement (52-57%) for Faecalibacterium prausnitzi, Shigellea/Escherichia and Bifidobacterium species (see table).

Conclusions

- 55% had dysbiosis at baseline and after 2 years
- 17% tested negative both times
- 15% got dysbiosis
- 13% lost it
- Some bacteria were very stable, while others were more unstable.
- Testing the stability may be of interest in future studies to treat specific disturbances in the gut microbiota.

	Comparison of DBT (n=33) vs NHD (n=10)		Comment	Agreement in abundance between test 1 and 2 (%) for DBT**
	Test 1	Test 2		
	P-value*			
Bacteria				
Ruminococcus albus/bromii	0.66	0.66		76
Ruminococcus gnavus	1.00	1.00		85
Faecalibacterium prausnitzii	0.07	0.24	DBT group lower at test 1	57
Lactobacillus	0.17	1.00		85
Streptococcus sanguinis and S. salivarius	1.00	0.61		85
Dialister invisus	0.73	**0.04**	DBT higher at test 2	67
Akkermansia muciniphilia	0.21	0.66		76
Bacteroides fragilis	0.45	0.28		67
Alistipes	1.00	1.00		82
Shigella/Escherichia	**0.02**	0.24	DBT lower at test 1	52
Bifidobacterium	**0.04**	1.00	DBT lower at test 1	52
Bacteroides/Prevotella	0.60	**0.01**	DBT higher at test 2	61
Firmicutes (Bacillli)	0.85	0.86		63
Firmicutes (Clostridia)	1.00	0.59		58
Proteobacteria	0.29	1.00		70

DBT = dysbiosis both times (2013 and 2015). NHD= never had dysbiosis. *Fisher's exact test. ** Abundance was measured semi-quantitatively as low, normal or high.

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CURRENT CATEGORY: Neurogastroenterology & Motility
PRESENTATION TYPE: AGA Institute Oral or Poster
PRESENTER: Jørgen Valeur
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Abstract

TITLE: Microbial DNA markers associated with response to a low FODMAP diet in patients with irritable bowel syndrome

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ABSTRACT BODY:

Abstract Body: Background: Dietary restriction of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) may relieve symptoms of irritable bowel syndrome (IBS). However, nutritional counselling is cumbersome, costly and time-consuming, and not all patients will benefit. In the present study, we aimed to explore whether microbial DNA markers may be used to identify a positive response to a low FODMAP diet in patients with IBS.

Materials and methods: Patients with IBS were recruited consecutively from our outpatient clinic to participate in a 4-week FODMAP restricted diet. Symptoms were evaluated by using the IBS severity scoring system (IBS-SSS), and response to diet was defined as > 50% decrease in IBS-SSS compared to baseline. Fecal samples were collected at baseline and analysed for microbial DNA by using the GA-map Dysbiosis Test (Genetic Analysis AS, Oslo, Norway).

Results: Sixty-one patients (54 F, 7 M) were included, of whom 32 (29 F; 3 M) were classified as responders and 29 (25 F; 4 M) were classified as non-responders. We assessed microbial DNA using 54 probes. Of those, 10 were significantly different between responders and non-responders (Table 1). Based on median values of responders for these markers, we constructed an index: Each participant was given a point when his/her value for each selected marker differed from the median cut-off value. These points were then summed up, giving a number from 0 to 10. The risk of being a non-responder was calculated using logistic regression. Those who scored 3 or more points using our index were 1.78 times more likely to be non-responders compared to those who scored lower (p = 0.002)

Conclusion: Our data suggest that microbial DNA markers may be a useful tool to select patients who are more likely to respond to a low FODMAP diet. Further studies are needed to validate these findings.

TABLE:

Table 1

Probe name	Responders (median signal)	Non-responders (median signal)	P-value
Bacteroides fragilis [s]	24.8	8.1	0.04
Acinetobacter [g]	187.3	177.2	0.02

Ruminiclostridium [g]	50.9	45.2	0.01
Clostridia [cl], Negativicutes [cl], Bacilli [cl]	497.4	617.1	0.02
Streptococcus III [g]	11.1	8.4	0.03
Actinomycetales [o]	5.8	8.8	0.02
Anaerotruncus [g]	76.8	86.6	0.004
Clostridiales [o]	274.3	288.6	0.004
Eubacterium II [g]	31.3	14.5	0.03
Shigella [g], Escherichia [g]	12.2	15.2	0.04
Abbreviations: s - species; g - genus; o - order; cl - class			

(No Image Selected)

Microbial characterization of pediatric inflammatory bowel disease and stratification into disease severity groups.

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Department of Pediatrics, Akershus University Hospital, Lørenskog, [2] Institute for Clinical Medicine, University of Oslo., Department of Gastroenterology Rikshospitalet, Oslo University Hospital, Genetic Analysis AS, Oslo, Epigen, Institute for Clinical Medicine; Campus AHUS, University of Oslo, [6]Department of Pediatrics, Oslo University Hospital, Norway

BACKGROUND AND AIM: Imbalance in the fecal microbiota with a reduction in biodiversity; dysbiosis, has been identified in inflammatory bowel disease (IBD) patients. Our aim was to study and compare the fecal microbiota in pediatric patients with newly diagnosed and untreated IBD with the microbiota of healthy children and pediatric patients with gastrointestinal symptoms but no IBD. Further, we aimed at studying the microbiota related to IBD subgroups and treatment.

METHODS: Fecal samples were collected from 235 children and adolescents. 110 had IBD, 80 had Crohn's disease (CD), 27 ulcerative colitis (UC) and 3 IBD unclassified, 50 were non-IBD symptomatic patients and 75 were healthy children between two and 18 years.

The microbiota was analysed using a 16s rRNA DNA based test with the GA-map technology, measuring probe signal intensity (PSI) of 54 DNA probes targeting 300 bacteria on different taxonomic levels.

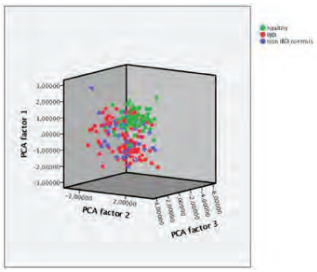
Using non-parametric methods, we identified six probes where the PSI was lower in IBD compared to non-IBD patients. For each of these six probes, IBD patients were given 1 point if their PSI was lower than the median PSI value of non-IBD patients. The points were summarized as a Score ranging from 0-6 points. Logistic regression was used to model possible associations between this Score and risk of having IBD.

RESULTS: **IBD and non- IBD patients compared with healthy children:** Majority of bacterial PSIs were significantly reduced in IBD and non-IBD patients ($p < 0.001$) compared to healthy controls.

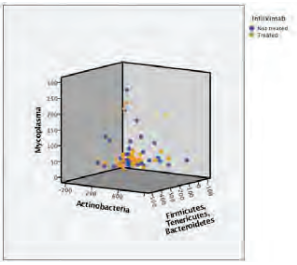
IBD (n=110) vs non- IBD symptomatic patients (n=50): IBD patients had reduced abundance of Firmicutes (*Eubacterium* p=0.006, *Holdemanella* p=0.038), *Tenericutes* and *Bacteroidetes* (*Parabacteroidetes* p=0.02), p=0.002, and *Bifidobacterium*, p=0.02, compared to non-IBD patients. Patients who reached 3 or more points using the Score were 2.2 times more likely to have IBD compared to non-IBD (OR=2.1, 95%CI 1.1-4.5, p=0.03).

CD vs UC: CD patients had lower abundance of *Mycoplasma* than UC patients, p=0.045.

Prognostic Factors: IBD patients with extensive disease (L3/E3) had more *Clostridiales* (*Ruminococcus gnavus*), p=0.02. CD patients with L3 had more *Proteobacteria* than patients with limited disease, p=0.04.
IBD patients who received TNF blockers, (64/110), had lower diversity at baseline for *Firmicutes*, *Tenericutes* (*Mycoplasma* p=0.009), and *Bacteroidetes*, p=0.015, compared to IBD patients who were treated with conventional medications (46/110).



Scatter plot showing microbial shifts/ dysbiosis in pediatric IBD and non-IBD patients compared to healthy children



Scatter plot illustrating differences in the microbiota in Infliximab vs conventional treated pediatric IBD patients.

CONCLUSION: Microbiota profiles may be of value for stratification of pediatric IBD into diagnostic and prognostic subgroups. A severe dysbiotic microbiota profile in newly diagnosed IBD is associated with a severe phenotype with more extensive disease and subsequent need of TNF blocker treatment.

Ref . Casén C et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther 2015.

Microbial characterization of paediatric inflammatory bowel disease and stratification into disease severity groups.

Christine Olbjørn[ab], Milada C Småstuen[c], Espen Thiis-Evensen[d], Britt Nakstad[ab], Jon B Rove[a], Torbjørn Lindahl[e], Christina Casén[e], Morten H Vatn[f], Gøri Perminow[g]
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Background: Imbalances in the faecal microbiota with a reduction in biodiversity; dysbiosis, has been identified in inflammatory bowel disease (IBD). Our aim was to study and compare the faecal microbiota in paediatric patients with newly diagnosed untreated IBD with the microbiota of healthy children and paediatric patients with gastrointestinal symptoms but no IBD. We also aimed at studying the microbiota related to IBD subgroups and treatment.
Methods: Faecal samples were collected from 235 children and adolescents. Eighty had Crohn's disease (CD), 27 ulcerative colitis (UC) and 3 IBD unclassified, 50 were non-IBD patients and 75 were healthy children between two and 18 years. The microbiota was analysed using a 16s rRNA DNA based test with the GA-map technology, measuring probe signal intensity (PSI) of 54 DNA probes targeting 300 bacteria on different taxonomic levels. The PSI from each probe was standardized using mean and standard deviations (SD) of the healthy children distribution and expressed as Z-scores. A Z score $\geq$2 SD was considered indicative of dysbiosis. We selected 5 probes where the PSI was lower in IBD compared to in non-IBD patients. Using median values of the distribution of non-IBD patients, IBD patients were given 1 point for each of these probes if their values were lower than this median. The scores were summarized, 5 points= max score. Logistic regression was used to model possible associations between this score and risk of having IBD.
Results: Most bacterial PSI were reduced in IBD and non-IBD patients ($p< 0.00$) compared to healthy controls. Patients who reached 3 or more points using the score were 2,1 times more likely to have IBD compared to non-IBD (OR=2.1, 95%CI 1.1-4.2, p=0.03). IBD patients had reduced abundance of Firmicutes (Eubacterium p=0.006, Holdemanella p=0.038), Tenericutes and Bacteroidetes (Parabacteroidetes p=0.02), p=0.002, and Bifidobacterium, p=0.02, compared to the non-IBD patients. CD patients had lower abundance of Mycoplasma (p=0.045) than UC patients. IBD patients with extensive disease (L3/E3) had more Clostridiales (Ruminococcus gnavus), p=0.02, and CD patients with L3 had more Proteobacteria, p=0.04, than patients with limited disease. IBD patients who later received TNF blockers, 64/110, had lower diversity at baseline for Firmicutes, Tenericutes (Mycoplasma p=0.009), and Bacteroidetes, p=0.015, compared to IBD patients who were treated with conventional medications, 46/110.
Conclusions: Microbiota profiles may be of value for stratification of paediatric IBD into diagnostic and prognostic subgroups. A severe dysbiotic microbiota profile in newly diagnosed IBD is associated with a severe phenotype with more extensive disease and subsequent need of TNF blocker treatment.

European Crohn's and Colitis Organisation 16-18 February 2017 P788 IBD Character

Microbiota related disease activity and distribution in subgroups of Inflammatory Bowel Disease

P. Ricanek[1], R. Kalla[2], Y. Ber[3], S. Vatn[1], L. Finnby[6], T. Lindahl[6], D. Bergemalm[4], J.D. Söderholm[5], J. Jahnsen[1], F. Gomollon[3], J. Halfvarson[4], J. Satsangi[2], C. Casén[6], M.H. Vatn[7], and the IBD-Character consortium.

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Aim of study

Knowledge about a patients' microbiota profiles might give useful information in diagnosing, early relapse prediction and to distinguish responders from non-responders to treatment. Fecal calprotectin (FCal) is used as a marker in diagnosis and follow-up of patients with inflammatory bowel diseases (IBD).

This IBD-Character study aims to evaluate any relationships between FCal and dysbiosis by analysing:

- Fecal microbiota profiles
- Microbial diversity
- Concentration of FCal

Methods

- Subjects: Treatment naive newly diagnosed IBD patients, symptomatic non-IBD patients and healthy controls
- Microbiota analysis: The microbiota was analysed using a 16s rRNA based test with the GA-map™ technology(1), measuring probe signal intensity of 54 DNA probes targeting >300 bacteria on different taxonomic levels. Dysbiosis was defined as non, mild or severe, in addition to analysis of specific microbial taxa.
- Fecal calprotectin: High FCal was defined as >100 µg/g (fCAL® ELISA, Bühlmann laboratories AG).
- Stool samples collected within 369 days prior to and within 14 days after diagnosis (=onset of treatment), and with no antibiotic treatment last two months, were included.

Results

- Data on dysbiosis, bacteria profiles and FCal were available in 41 CD, 58 UC, 8 IBD-U patients, and 129 symptomatic non-IBD and 45 healthy controls (n=281).
- There was a relationship between FCal and dysbiosis in UC patients (p=0.0249, ANCOVA), which was not the case for CD and the control groups.

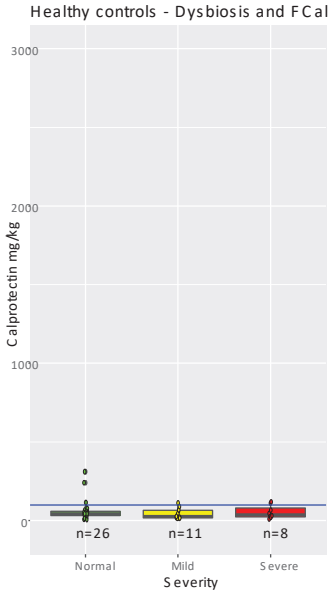

Figure 1. Healthy controls n= 45. The figure shows there is no relationship between degree of dysbiosis and concentration of FCal.

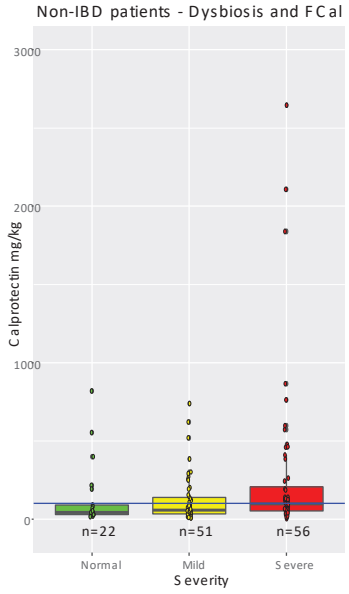

Figure 2. Symptomatic non-IBD controls, n= 129. The figure shows there is no relationship between degree of dysbiosis and concentration of FCal

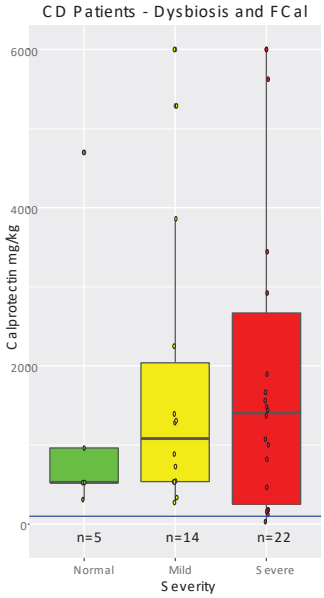

Figure 3. CD patients, n= 41. The figure shows there is no relationship between degree of dysbiosis and concentration of FCal.

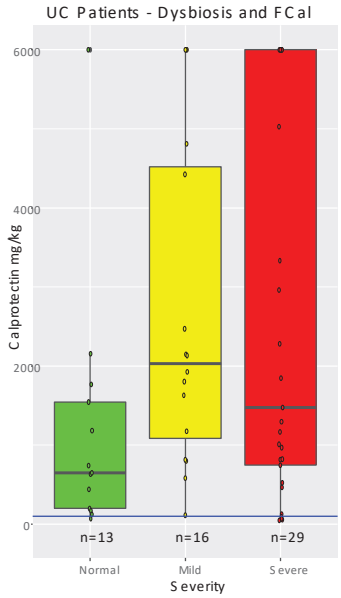

Figure 4. UC patients, n= 58. The figure shows there is a relationship between degree of dysbiosis and concentration of Fcal (p=0.0249).

Conclusions

- Significant relationship between FCal and dysbiosis in the UC group
- No significant relationship between FCal and dysbiosis in the CD patient group or the control groups
- The data demonstrate a diagnostic potential for a microbiota test in IBD

1 Casén C et al Aliment Pharmacol Ther 2015;42:71-83

Accepted abstract (oral) PIBD Sept 2017; Dr Christine Olbjørn, NO

Faecal microbiota dysbiosis in paediatric inflammatory bowel disease persists after therapy

Objectives and study: Imbalances in the faecal microbiota with a reduction in biodiversity; dysbiosis, have been identified in inflammatory bowel disease (IBD). Our aim was to study and compare the faecal microbiota in paediatric patients with newly diagnosed untreated IBD with the microbiota of healthy children and paediatric patients with gastrointestinal symptoms but no IBD. We also related the microbiota profiles to IBD subgroups and later treatment.

Methods: Faecal samples were collected from 235 children and adolescents. Eighty had Crohn's disease (CD), 27 ulcerative colitis (UC) and 3 IBD unclassified, 50 were non-IBD symptomatic patients and 75 were healthy children between age two and 18 years. The microbiota was analysed using a 16s rRNA DNA based test with the GA-map technology (Genetic Analysis AS, Oslo, Norway) measuring probe signal intensity (PSI) of 54 bacterial DNA probes covering 300 bacteria on different taxonomic levels. We compared the PSI values of the three groups, healthy children, IBD patients and non-IBD patients. Using non-parametric methods, we selected six probes where the PSI was lower in IBD compared to non-IBD patients. For each of these six probes, IBD patients were given 1 point if their PSI was lower than the median PSI value of non-IBD patients. The points were summarized as a Score ranging from 0-6 points. Logistic regression was used to model possible associations between this Score and risk of having IBD.

Results: Most bacterial PSIs were reduced in IBD and non-IBD patients ($p< 0.001$) compared to healthy controls. IBD patients had reduced abundance of Firmicutes (Eubacterium p=0.006, Holdemanella p=0.038, Streptococcus p=0.046), Tenericutes and Bacteroidetes (Parabacteroidetes p=0.02), p=0.002, and Bifidobacterium, p=0.02, compared to the non-IBD symptomatic patients. CD patients had an overall lower diversity compared to UC patients, but this did not reach statistical significance except for Mycoplasma, where CD patients had a slightly lower abundance (p=0.045). IBD patients with extensive disease (L3/E3 according to the Paris classification) had more Clostridiales (Ruminococcus gnavus), p=0.02, and CD patients with L3 had more Proteobacteria, p=0.04, than patients with limited disease. Upper gastrointestinal manifestations in CD patients were associated with higher PSIs of the Firmicutes Bacilli, Clostridia and Veillonella (p=0.008) and the Proteobacteria Helicobacter p=0.008, and lower PSIs of the Proteobacteria Shigella and Escherichia (p=0.05). For IBD patients who later received biologic therapy with TNF blockers, 64 (58%), had lower diversity at baseline for Firmicutes, Tenericutes (Mycoplasma p=0.009), and Bacteroidetes, p=0.015, compared to IBD patients who were treated with conventional (exclusive enteral nutrition, immunomodulators, corticosteroids, 5-Aminosalicylic acids) medications, 46 (42%). Patients who reached 3 or more points using the Score were 2.2 times more likely to have IBD compared to non-IBD (OR=2.2, 95%CI 1.1-4.5, p=0.027).

Conclusion: Microbiota profiles may be used to stratify paediatric IBD into diagnostic and prognostic subgroups. A severe dysbiotic microbiota profile in newly diagnosed paediatric IBD is associated with a phenotype with more extensive disease and subsequent need of TNF blocker treatment.

Faecal microbiota in paediatric inflammatory bowel disease before and after therapy.

C. Olbjørn, M. Småstuen, E. Thiis-Evensen, B. Nakstad, C. Casén, M. Vatn, G. Perminow

Presentation Number
OP157

Introduction

Imbalances in the faecal microbiota with a reduction in biodiversity; dysbiosis, have been reported in inflammatory bowel disease (IBD).

Aims & Methods

Our aim was to study and compare the faecal microbiota in paediatric patients with newly diagnosed untreated IBD with the microbiota of healthy children and paediatric patients with gastrointestinal symptoms but no IBD. We also wanted to study microbiota changes in IBD patients one year after initiation of treatment.

Faecal samples were collected from 235 children below 18 years of age. IBD was diagnosed in 110 patients, 80 had Crohn's disease (CD), 27 had ulcerative colitis (UC) and 3 had IBD unclassified. Fifty patients had gastrointestinal symptoms but no IBD; non-IBD symptomatic patients, and 75 were healthy children. Of the IBD patients, 31 (9 with UC and 22 with CD) had repeated faecal analysis one year after therapy, 16 (52%) had been treated with infliximab. The microbiota was analysed at baseline and follow-up using a 16s rRNA DNA based test with the GA-map technology, measuring probe signal intensity (PSI) of 54 DNA probes targeting 300 bacteria on different taxonomic levels.

Results

At baseline the majority of bacterial PSIs were reduced in IBD and non-IBD patients (both $p< 0.001$) compared to healthy controls. IBD patients had significantly reduced abundance of various Firmicutes $p<0.01$ (*Eubacterium rectale, Eubacterium biforme*), Bacteroidetes $p=0.02$ (*Parabacteroidetes)*, and of *Bifidobacterium* $p=0.02$, compared to non-IBD patients.

In the 31 IBD patients with repeated faecal samples the microbiota was more dysbiotic after therapy, regardless of IBD type and whether the IBD patient had received infliximab or not, with less abundance of the Clostridia species *Dorea spp., Lachnospiraceae* and *Eubacterium hallii* ($p<0.001$). Compared to healthy and non-IBD patients the microbiota composition after treatment had significantly ($p<0.001$) less abundance of *Akkermansia muciniphila, Bacteroides spp., Prevotella spp.* and *Veillonella spp.* besides higher abundance of *Streptococcus sanguinis, Atopobium rimae and* pro-inflammatory Proteobacteria *(Shigella spp., Escherichia spp.)*

Conclusion

The faecal microbiota composition is significantly different in paediatric IBD and non-IBD symptomatic patients compared to healthy children and may be of value in diagnosing

IBD. A severe dysbiotic microbiota profile seem to persist and even worsen after treatment in paediatric IBD patients regardless of treatment with infliximab or not.

References

1. Casén C et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther. 2015 Jul;42(1):71-83.

25th uegweek United European Gastroenterology Week October 28 – November 1, 2017 Barcelona, Spain

P# 5548

IBD Character

Dysbiosis of the gut microbiota in relation to disease activity in IBD

P. Ricanek[1], R. Kalla[2], Y. Ber[3], S. Vatn[1], M.K Karlsson[6], L. Finnby[6], D. Bergemalm[4], A. Carstens[4], J.D. Söderholm[5], J. Jahnsen[1], F. Gomollon[3], J. Halfvarson[4], J. Satsangi[2], C. Casén[6], M.H. Vatn[7], and the IBD-Character consortium.
[1]Lørenskog/NO, [2]Edinburgh/GB, [3]Zaragoza/ES, [4]Örebro/SE, [5]Linköping/SE, [6]GA, Oslo/NO, [7]UiO/NO

Aim of study

- Express alterations in the gut microbial community by the term 'dysbiosis'.
- Explore associations between measures of gut microbiota and clinical as well as inflammatory disease activity.

Methods

- Microbiota analysis: The GA-map™ Dysbiosis Test[1] utilises the 16S rRNA gene for bacteria determination.
- Dysbiosis was measured as non, mild or severe.
- Clinical disease activity: HBI (CD) and SCCAI (UC).
- Inflammatory disease activity: Fecal calprotectin (fCAL® ELISA, Bühlmann laboratories AG) and CRP.

Results

- Increasing dysbiosis severity in UC, CD and non-IBD patients yielded lower abundance of *Faecalibacterium prausnitzii*, and higher abundance of Proteobacteria.
- *Bifidobacterium* yielded lower abundance with increased dysbiosis severity in UC and non-IBD patients, and in combination with elevated levels of fCAL and/or CRP in UC patients.
- In the healthy controls, increasing dysbiosis severity yielded higher abundance of Proteobacteria.

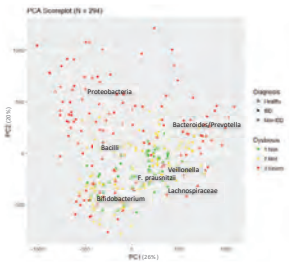

Figure 1. PCA scores for first 2 principal components for the study cohort (N = 294) based on 54 DNA probes.

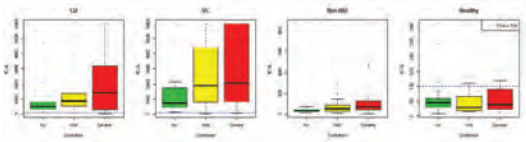

Figure 2. Boxplots for dysbiosis severity (x-axis) and fCAL (y-axis). The results show association between fCAL and dysbiosis in non-IBD (P=0.04), trend for CD and UC, and no association for healthy.

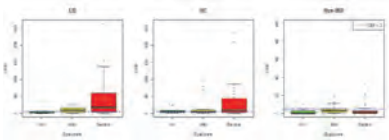

Figure 3. Boxplots for dysbiosis severity (x-axis) and CRP (y-axis). The results show association between CRP and dysbiosis in CD (P=0.02), while not for UC and non-IBD.

Table 1. Study cohort

Diagnosis	N	Age median (min-max)	Gender (female)	fCAL median	Dysbiosis frequency
CD	57	29 (18-66)	60%	1005	84%
UC	80	35 (18-69)	46%	1700	84%
IBD-U	12	33.5 (25-67)	58%	2361	67%
Non-IBD	100	34 (18-63)	54%	54	86%
Healthy	45	25 (19-66)	64%	43	42%
Total	294	31 (18-69)	55%		

Table 2. P-values from ANOVA for dysbiosis severity and disease activity

Diagnosis	fCAL	CRP	HBI/SCCAI
CD	0.2	0.02	0.2
UC	0.08	0.2	0.3
IBD-U	0.005	0.4	
Non-IBD	0.04	0.1	
Healthy	0.6		

Conclusions:

- Relationship between dysbiosis and fCAL in sub-groups of IBD and non-IBD, in CD patients also with CRP.
- *Bifidobacterium* yielded lower abundance with increased dysbiosis severity in UC and non-IBD patients.
- Gut bacteria profiles and abundance may be used to differentiate between severity in UC and CD patients, as a non-invasive tool to monitor disease activity in IBD.

[1]Casén *et al*. Aliment Pharmacol Ther 2015; 42: 71–83

Effect of smoking on gut microbiota in a cohort of normal Italian subjects; a cross-country population study

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KEYWORDS: Microbiota, gut, smoking

Introduction

The composition of the gut microbiota is relatively stable throughout adult life, but the microbiome is susceptible to the influence of factors such as of bacterial infections, antibiotics, diet, and smoking, and can thereby be transiently or permanently altered.

Methods

To characterize dysbiosis, we conducted 16S rRNA analysis using GA-map™ Dysbiosis Test[1] of fecal samples collected from normal Italian adults residing in 3 regions of Italy (N = 78, 50% female, median age 55). Participants were recruited from subjects coming to the clinic for colonoscopy in connection to the national screening program, with no abnormal findings. Each participant also completed a 16-question questionnaire.

Chi-square test was used to determine differences in proportions, with $p < 0.05$ for significance.

Results

In total 60% (47/78) of normal Italian adults were determined to be normobiotic, 36% (28/78) were determined to have mild dysbiosis, and 4% (3/78) were determined to have severe dysbiosis. Among 27 smoking subjects, 15 (56%) were determined to be dysbiotic, while only 16 (31%) of 51 non-smoking subjects were dysbiotic. The proportion of dysbiosis between the two groups was found to be significant ($p = 0.04$). We observed high variability in the profiles of fecal microbiota among the Italian adults. However, smoking subjects tended to have reduced markers for Actinobacteria (mainly the genus Bifidobacterium), Firmicutes, and Lactobacillus, while increased markers for Bacteroidetes.

Conclusion

We used the GA-map™ Dysbiosis Test to determine and characterize dysbiosis in normal Italian adults. The present study showed that the composition of the fecal microbiota of normal Italian adults, while highly variable, was strongly associated with subjects' smoking habits. Altogether, our results indicate a 40% proportion of dysbiosis in normal Italian adults, which may possibly be caused by environmental factors such as dietary or smoking habits, as we observe a 25% higher proportion of dysbiosis among smokers as compared to non-smokers.

References

1 Casén C, *et al*. (2015) Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther.; 42(1):71-83

European Crohn's and Colitis Organisation

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IBD Character

Fecal microbiota in newly diagnosed Crohn's disease and its relation to treatment escalation

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Background

Crohn's disease (CD) is a chronic inflammatory disease which can affect any part of the gastrointestinal tract. The treatment aims at inducing and maintaining remission based on clinical symptoms, endoscopic appearance and biochemical markers. Some patients have a mild disease course whilst other develop a refractory disease or complicated disease behavior, due to a stricturing and/or penetrating phenotype, demanding treatment escalation.

Tools for predicting the disease course are limited. Identification of microbiota signatures, might be a tool for identification of patient with a poor prognosis and need of treatment escalation.

Methods

Fecal samples were obtained at diagnosis from 33 newly diagnosed CD patients in the IBD-character cohort (Table 1). Disease course and treatment were followed up to 5 years after inclusion. The cohort was stratified into 2 groups based on disease progression demanding treatment escalation or not (introduction of biologic treatment and/or surgery after initial treatment).

Fecal microbiota composition was assessed using the GA-map™ Dysbiosis Test [1], which comprises 54 bacterial probes that target variable regions V3 to V7 of the bacterial 16S rRNA gene (Figure 1).

Comparisons between the groups were made using the Wilcoxon test.

1 Casén C et al Aliment Pharmacol Ther 2015;42:71-83

Table 1. Study samples.

Treatment escalation	n	Age years median (range)	Gender	FCal mg/kg median (range)
No	21	30 (22-78)	15F/6M	540 (32-5627)*
Yes	12	28.5 (18-50)	4F/8M	1564 (148-3862)*

*1 patient with measurement >6000 mg/kg

Figure 1. Target variable regions in the bacterial 16S rRNA gene utilized by the GA-map™ Dysbiosis Test compared to Illumina.

Results

Among 33 CD patients, 28 (85%) were classified as dysbiotic (11 mild and 17 severe). A total of 12 (36%) patients required treatment escalation.

At the time of diagnosis, the dysbiosis index, did not discriminate between patients who did or did not require treatment escalation.

Table 2. Distribution of detected dysbiosis severity.

Treatment escalation	Dysbiosis		
	No	Mild	Severe
No	4	7	10
Yes	1	4	7

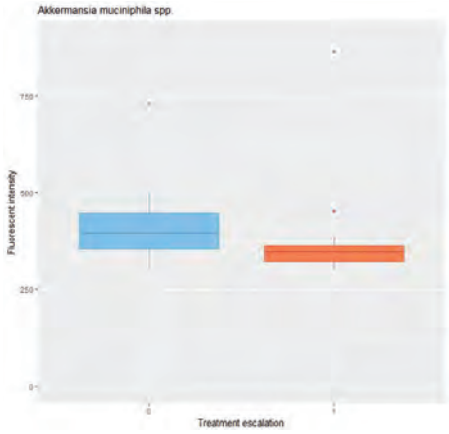

Figure 2. Fluorescent intensity of *Akkermansia muciniphila*. The bacterial probe intensity was found to be reduced in CD patients who required treatment escalation (1, x-axis) compared to those who did not (0, x-axis) (p = 0.04).

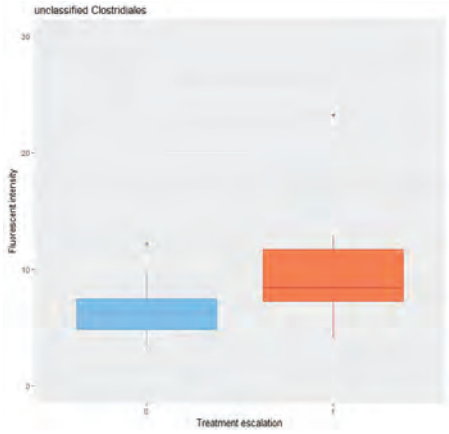

Figure 3. Fluorescent intensity of an unclassified Clostridiales. The bacterial probe intensity was found to be significantly increased in CD patients who required treatment escalation (1, x-axis) compared to those who did not (0, x-axis) (p = 0.03).

Conclusions

- An aggressive disease course exhibited a decreased intensity of *Akkermansia muciniphila* and an increased intensity of Clostridiales already at diagnosis
- Our results might point to the relevance of these taxa for the discovery of predictive biomarkers that can be used to support tailored treatment in CD

European Crohn's and Colitis Organisation

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IBD Character

Fecal microbiota in treatment-naïve ulcerative colitis and its relation to treatment escalation

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[1]Akershus University Hospital/NO, [2]Genetic Analysis AS, Oslo/NO, [3]Örebro University/SE, [4]Ersta Hospital/SE, [5]University of Oslo/NO

Background

Ulcerative colitis (UC) is a chronic inflammatory disease affecting the large intestine. The disease course varies from an indolent disease to an aggressive disease, requiring early introduction of biologics and colectomy in treatment refractory individuals.

There is a clinical need of biomarkers that can be used to predict the future disease course already at diagnosis. Microbiota signatures might be of help in this respect and could potentially become a tool for the implementation of personalized medicine.

Methods

Fecal samples were collected at diagnosis from 47 treatment-naïve UC patients from Norway and Sweden in the IBD-character cohort. Extent of inflammation was defined according to the Montreal classification.

Table 1. Study samples.

Treatment escalation	n	Age years median (range)	Gender	FCal mg/kg median (range)
No	41	35 (18-77)	21F/20M	1394 (70-6001)*
Yes	6	25.5 (18-60)	4F/2M	3318 (58-6001)**

*5 patients with measurement >6000 mg/kg. **3 patients with measurement >6000 mg/kg.

Fecal microbiota composition was assessed using the GA-map™ Dysbiosis Test [1], which comprises 54 bacterial probes that target variable regions V3 to V7 of the bacterial 16S rRNA gene (Figure 1).

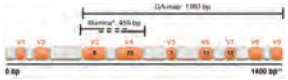

Figure 1. Target variable regions in the bacterial 16S rRNA gene utilized by the GA-map™ Dysbiosis Test compared to Illumina.

Patients were followed prospectively for up to 5 years and information on treatment escalation and surgery was collected. Patients were categorized into 2 groups based on need of treatment escalation, defined as introduction of biologics and/or colectomy during the study period.

Differences between groups were compared by using the Wilcoxon test.

Results

Among 47 UC patients, 38 (81%) were classified as dysbiotic. A total of 6 (13%) patients required treatment escalation. Patients with extensive colitis (E3) seemed to be more likely to require treatment escalation than patients with left-sided colitis (E2) or proctitis (E1) [OR = 4.8, 95% CI (0.78-30.0); p = 0.09)]. No significant association was found between dysbiosis severity and treatment escalation during follow-up ($p > 0.05$).

Table 2. Distribution of detected dysbiosis severity and UC subtypes.

Treatment escalation	Dysbiosis			UC subtype		
	No	Mild	Severe	E1	E2	E3
No	8	11	22	18	11	12
Yes	1	1	4	0	2	4

Results

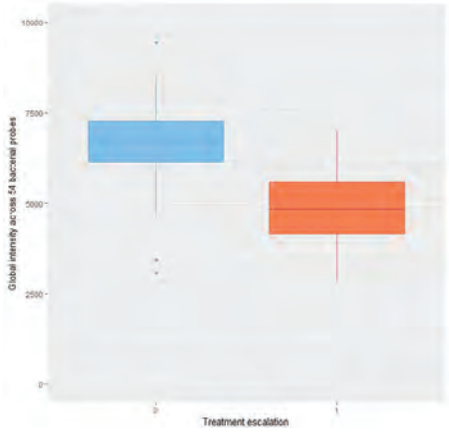

Figure 2. The global intensity of all 54 bacterial probes. Significantly lower in patients who required treatment escalation (1) compared to patients who did not require escalation (0) (p = 0.008).

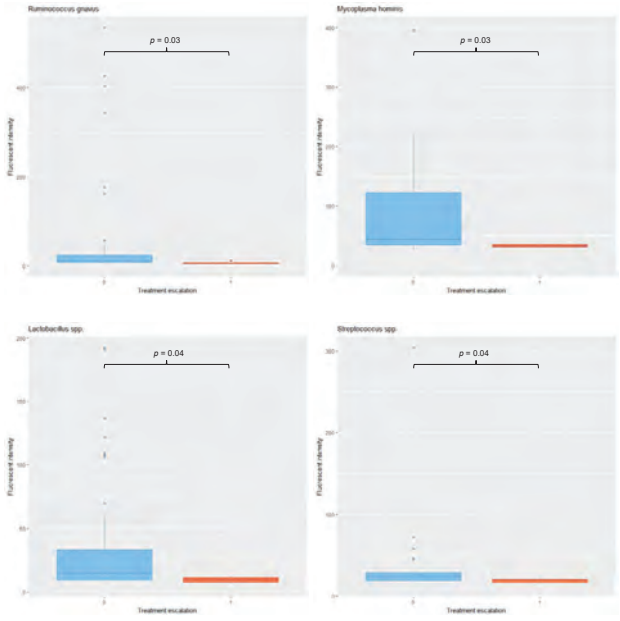

Figure 3. The measured probe intensity of *Ruminococcus gnavus* (a), *Mycoplasma hominis* (b), *Lactobacillus* spp. (c), and *Streptococcus* spp. (d). Significantly lower in patients who required treatment escalation (1) compared to patients who did not require escalation (0).

Conclusion

Decreased signal intensity of *Ruminococcus gnavus*, *Mycoplasma hominis*, *Lactobacillus* spp., and *Streptococcus* spp. at diagnosis of UC is associated with a more aggressive disease

1 Casén C et al Aliment Pharmacol Ther 2015;42:71-83

Could a test discriminate between the "good" and "bad" faecal dysbiosis? A study in subjects with morbid obesity.

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Introduction: Faecal dysbiosis (an imbalance in the faecal microbiota) has been associated with disorders such as obesity and Irritable bowel syndrome (IBS), and with the use of non-nutritive sweeteners (NNS) ("bad" dysbiosis) and with the use of Metformin, which has been considered as a favourable effect of the drug ("good" dysbiosis). Aims: Study the diagnostic properties of a commercially available test for faecal dysbiosis in subjects with morbid obesity, and explore the test's ability to discriminate between different types of dysbiosis.

Methods: Consecutive subjects with morbid obesity (BMI ≥ 40 kg/m^2 or ≥35 kg/m^2 with obesity-related complications) referred to a hospital unit for obesity were included in this cross-sectional study. A medical history was taken, the dietary intake was assessed with a food frequency questionnaire, gastrointestinal complaints including IBS were scored according to the Rome III criteria and the severity with Irritable Bowel Severity Scoring System (IBSSS), a physical examination was performed, a blood sample was collected, and faecal dysbiosis was assessed with GA-map^{TM} Dysbiosis Test (Genetic Analysis AS, Oslo, Norway). The results of the dysbiosis test were given as Dysbiosis Index (DI) 1-5, values ≤ 2 = no dysbiosis. To discriminate between the dysbiosis associated with NNS and Metformin, an alternative dysbiosis index (ADI) (score – 10; 10) was constructed based on exploratory analyses of the changes in a selection of the 54 DNA probes used in the test. Positive ADI scores were associated with the "good" dysbiosis and negative with the "bad" dysbiosis.

Results: Out of 350 consecutive subjects, 90 (76 women and 14 men with a mean age of 44.7 years (SD 8.6) and BMI 41.8 kg/m2 (SD 3.6)) were included. Dysbiosis was present in 59 (66%) of the subjects with a mean DI score of 3.0 (SD 1.3). The DI scores 1-5 were present in 16 (18%), 15 (17%), 30 (33%), 13 (14%), and 16 (18%) subjects respectively. Dysbiosis was associated with smoking (p=0.046), coffee (p=0.026), Metformin (p=0.002) and NNS (p<0.001), but not with IBS or IBSSS. ADI was positively associated with Metformin (p=0.002) (the "good" dysbiosis), and negatively associated with NNS (p=0.001) and IBSSS (p=0.004) (the "bad" dysbiosis).

Conclusion. The test demonstrated the expected associations between dysbiosis and NNS and Metformin, but not with IBS or IBSSS. The ADI showed the significant differences between the dysbiosis associated with NNS and IBSSS (the "bad" one), and with Metformin (the "good" one). Research should focus on types of dysbiosis and not only on dysbiosis per se.

Ehealth: Does faecal microbial dysbiosis correlate with disease activity measures, faecal calprotectin and symptom scores in adult patients with inflammatory bowel disease

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Introduction

There is increasing evidence substantiating that intestinal microbial dysbiosis in Inflammatory bowel disease (IBD) plays a role in the pathogenesis and progression hereof. Dysbiosis is poorly understood and therefore not yet considered for clinical use in optimizing individualized treatments of IBD.

The aim of the study was to characterize the gut microbiome of IBD patients in a consecutive eHealth cohort, and correlate the microbiome, a dysbiosis index to conventional disease activity measures to better understand and interpret the microbiome in IBD.

Methodology

During 1 year, 120 consecutive IBD patients in any IBD therapy were enrolled in the web-outpatient clinic at North Zealand University Hospital, Denmark to monitor for disease activity in an integrated web-app with Constant care ® and CalproSmart™ That consists of an algorithm cumulating SCCAI/HBI and calprotectin into a total inflammation burden score (Figure 1). A sub-group of patients consented to provide repeated faecal samples while screening for disease activity on constant care.
The faecal microbiome was analysed in two different ways:

1. The microbial dysbiosis index (DI); 1-2 normo-dysbiosis, 3-5 dysbiosis, (GA-map®~~™~~, Genetic Analysis AS ~~INC~~, Norway)[1]
2. V3-V4 16S rRNA amplicon-based sequencing, Illumina, SSI INC.

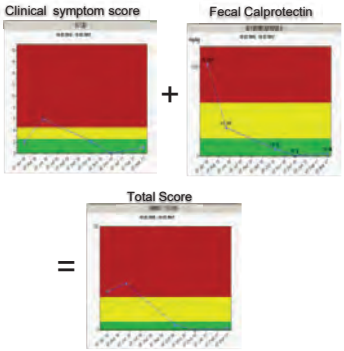

Figure 1. noh.constant-care.dk traffic light disease algorithm

Results

78 patients provided faecal samples for microbiome analysis. 11 (14%) were diagnosed with CD (n=36 samples), 63 (81%) with UC (n=230) and 4 (5%) with IBDU (n=22). Baseline characteristics can be found in table 1.

Median (IQR) for the following disease activity variables throughout the cohort
FC 82 (28-455), SCCAI 1 (0-2), HBI 3 (1-9) and DI 3 (2-4).

GA-map®~~™~~ DI Spearman correlations with disease activity measures:

FC and DI: rho= 0.24, p<0.01
DI and SCCAI: rho= 0.17, p=0.01
DI and HBI: rho= 0.25, p=0.17

Table 1. Baseline characteristics, microbiome cohort

	[illegible]	UC	CD	IBDU
Total	78 (100%)	63 (80.8)	11 (14.1)	4 (5.1)
Sex				
Male	41 (52.6)	32 (50.8)	7 (63.6)	2 (50.0)
Female	37 (47.4)	31 (49.2)	4 (36.4)	2 (50.0)
Mean body mass index (SD; kg/m²)	25.7 (4.7)	25.8 (4.9)	26.3 (4.3)	23.2 (2.3)
Smoking				
Current	6 (7.7)	4 (6.3)	1 (9.1)	1 (25.0)
Former	44 (56.4)	37 (58.7)	6 (54.5)	1 (25.0)
Never	28 (35.9)	22 (34.9)	4 (36.4)	2 (50.0)
Mean age at inclusion (SD; years)	48.7 (14.7)	48.4 (13.8)	51.5 (17.9)	46.0 (22.3)
Mean age at diagnosis (SD; years)	36.8 (13.7)	37.0 (12.5)	34.8 (18.2)	39.5 (21.4)
UC extent				
E1, proctitis		21 (33.3)		
E2, left side		25 (39.7)		
E3, extensive		17 (27.0)		
CD location				
L1, small bowel			1 (9.1)	
L2, colonic			1 (9.1)	
L3, ileo-colonic			9 (81.8)	
CD behavior				
B1, inflammatory			8 (72.7)	
B2, stricturing			3 (27.3)	
B3, penetrating			0	
Previous surgery	6 (7.7)	0	6 (54.5)	0
Median IBD duration (IQR; years)	8.5 (4.0-16.5)	7.0 (4.0-16.0)	15 (6.0-29.0)	6.5 (1.8-12.8)
Medical treatment				
None	12 (15.4)	7 (11.1)	4 (36.4)	1 (25.0)
5ASA	43 (55.1)	39 (61.9)	1 (9.1)	3 (75.0)
Immunosuppressive	5 (6.4)	2 (3.2)	3 (27.3)	0
Biological	3 (3.8)	2 (3.2)	1 (9.1)	0
Combination therapy	15 (19.2)	13 (20.6)	2 (18.2)	0
Education				
Short	6 (7.7)	6 (9.5)	0	0
Student	4 (5.1)	3 (4.8)	0	1 (25.0)
Medium	54 (69.2)	42 (66.7)	9 (81.8)	3 (75.0)
Higher/Academic	14 (17.9)	12 (19.0)	2 (18.2)	0
Occupation				
Yes	64 (82.1)	53 (84.1)	8 (72.7)	3 (75.0)
No	14 (17.9)	10 (15.9)	3 (27.3)	1 (25.0)
Patient reported Co-morbidities				
None	33 (42.3)	31 (49.2)	2 (18.2)	0
1	32 (41.0)	20 (31.7)	9 (81.8)	3 (75.0)
>1	11 (14.1)	11 (17.5)	0	0
Missing	2 (2.6)	1 (1.6)	0	1 (25.0)

Data are expressed as number (%), median (IQR) or mean (SD).

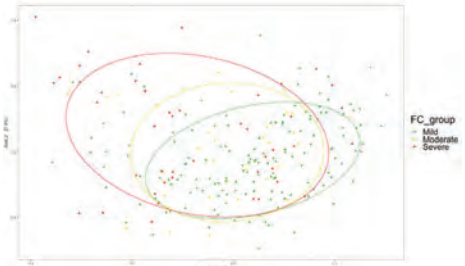

Figure 2. PCoA of prokaryotic microbime composition indicates relation to FC. ANOSIM R=0.14, p=0.001, SSI.

Based on V3-V4 16S rRNA sequencing data 3 clusters (PCoA) according to FC values categorized as; remission (Green, 0-200 mg/kg), moderate activity (Yellow, 200-599 mg/kg) and severe activity (Red, >599 mg/kg) showed a trend towards separation in these 3 groups, ANOSIM R=0.15, P=0.001 (Figure 2). No clear clusters were observed in regards to HBI and SCCAI.

Summary and Conclusions

FC and SCCAI showed relatively small but significant correlations with DI (Dysbiosis Index). 16S rRNA sequencing data showed a trend in separating FC between mild, moderate and severe inflammation. Further bioinformatic analyses are awaiting on the individual longitudinal data in relation to disease course and changes in disease activity.

Disclosure

Funding for FC home testing kits received from Calpro INC, Norway.

[1]Casén C et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. ATP 2015.

UEG Week Vienna October 20-24, 2018 **P1637**

Characterization of gut microbiota in a cohort of normal Italian subjects; results from a cross-country population study

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Introduction

The composition of intestinal microbiota is gaining importance in human health studies. There is increasing evidence that bacteria play a role in disease etiology. The composition of the gut microbiota is relatively stable throughout adult life, but can be transiently or permanently altered as a result of bacterial infections, antimicrobial therapy, surgery, lifestyle, and a long-term change in diet.

Methods

Fecal samples were collected from normal adults residing three regions of Italy (Milan, Rome, Palermo), recruited from clinics for colonoscopy in connection to the national screening program, with no abnormal findings. Each participant completed a 16-question questionnaire. Table 1 presents the study cohort.

For detection and characterization of dysbiosis, we conducted 16SrRNA gene analysis using the GA-map® Dysbiosis Test[1]. The test is composed of 54 pre-selected highly specific 16S rRNA gene-targeted single nucleotide primer extension (SNuPE) probes, detecting at least 300 bacteria at different taxonomic levels. The test reports a Dysbiosis Index (DI), where values 1-2 indicate normobiosis, and 3-5 indicate dysbiosis.

Fecal Calprotectin (FCal) analysis was performed using BÜHLMANN fCAL® ELISA with a cut-off of ≤200mg/kg.

Chi-square test was used to determine differences in proportions, with $p < 0.05$ for significance.

Table 1. Study cohort

Site	N	Age*	Gender, male	FCal*	Smokes, yes
Milan	36	51.5 (39-73)	39%	42 (8-190)	36%
Roma	34	57 (24-66)	50%	31.5 (6-169)	41%
Palermo	8	52.5 (46-68)	100%	42 (18-123)	0%
ALL	78	55 (24-73)	50%	37 (6-190)	35%

*Median (range)

Results

Of 78 normal Italian adults, 60% were determined to be normobiotic, 36% were determined to have mild dysbiosis, and 4% were determined to have severe dysbiosis (Figure 1, Table 2). However, no subjects were found to have the highest degree of dysbiosis with DI=5.

Overall, 56% of smoking subjects were determined to be dysbiotic (Figure 2), while the proportion was 31% in 51 non-smoking subjects ($p = 0.04$). No significant difference in proportion of dysbiosis was found between sites ($p > 0.1$) or gender ($p = 0.8$).

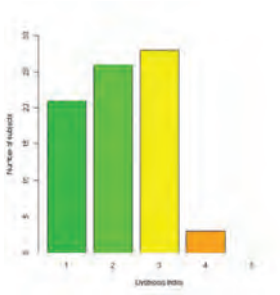

Figure 1. Distribution of DI in normal Italian adults (N=78).

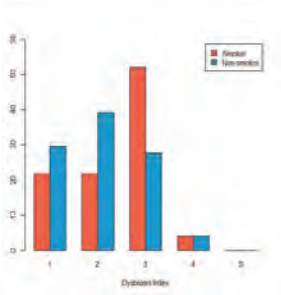

Figure 2. Percentage of DI between smokers and non-smokers.

Table 2. Test results

Site	Dysbiosis Index (DI) 1	2	3	4	5	Not Dysbiotic	Median DI
Milan	9	12	15			58%	2
Roma	11	12	9	2		68%	2
Palermo	1	2	4	1		38%	3
ALL	21	26	28	3	0	60%	2

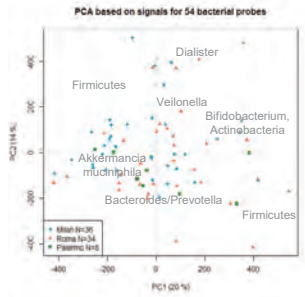

Figure 3. We observed high variability in the profiles of fecal microbiota among the Italian adults. The profiles were generally dominated by Actinobacteria (mainly the genus *Bifidobacterium*), Firmicutes (with diverse representation from numerous genera), Verrucomicrobia (*Akkermansia muciniphila*), and Bacteroidetes (mainly *Bacteroides* and *Prevotella*).

Conclusions:

- Fecal microbiota composition of normal Italian adults was not associated with area of residence or gender
- Smoking was found to be associated with dysbiosis
- Altogether, our results indicate a 40% proportion of dysbiosis in normal Italian adults, which may possibly be caused by environmental factors such as dietary or smoking habits, as we observe a 25% higher proportion of dysbiosis among smokers as compared to non-smokers

[1]Casén *et al.* Aliment Pharmacol Ther 2015; 42: 71–83

Are non-nutritive sweeteners obesogenic by altering the faecal microbiota and their metabolites?

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Introduction: This study explored changes in the faecal microbiota and short-chain fatty acids (SCFA) related to intake of non-nutritive sweeteners (NNS).

Methods: The study included patients aged 18 - 60 years with morbid obesity (BMI > 40 kg/m^2 or > 35 kg/m^2 with obesity-related complications). The diet was assessed with a validated food frequency questionnaire. One unit NNS was 100 ml beverage with NNS or 2 tablets/teaspoons NNS. The relative abundance of 39 faecal bacteria markers (scores – 3 to 3) was assessed with GA-map® dysbiosis test (Genetic Analysis, Oslo Norway). Acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, caproic, and iso-caproic acids in faeces were analysed with gas chromatography. The associations between NNS and SCFA and the bacteria were analysed with regression analyses with age, gender, starch in the diet and Metformin as covariates and reported as the unstandardized coefficients B with 95%CI and p-values.

Results: Data from 14(16%) men and 75(84%) women with a mean age of 44.6 years (SD 8.7) and BMI 42 kg/m^2 (SD 3.6) were analysed. Intake of NNS (mean and median) was 7.5 and 3.2 (SD 10; range 0-43) units respectively. The amounts (median and range) of acetic, propionic, butyric and valeric acids were 16.4 (2.9 – 67.9), 5.2 (1.3 – 25.6), 5.6 (1.0 – 34.5) and 0.8 (0.0 – 5.1) mmol/kg wet weight respectively. NNS was negatively associated with butyric acid (B: -0.159; 95%CI: -0.280 to -0.037; p=0.011), valeric acid (B: -0.022; 95%CI: -0.043 to -0.002; p=0.029) and *Faecalibacterium prausnitzii* (B: -0.056; 95%CI: -0.103 to -0.009; p=0.019), and positively associated with *Bacteroides fragilis* (B: 0.074; 95%CI: 0.025 to 0.122; p=0.003), *Ruminococcus gnavus* (B: 0.069; 95%CI: 0.009 to 0.128; p=0.024) and Streptococcus spp (B: 0.093; 95%CI: 0.036 to 0.150; p=0.001).

Conclusion: Butyrate has antiobesogenic effects and obesity has been associated with an increased content of gut Firmicutes. The findings of reduced faecal butyrate, reduced *Faecalibacterium prausnitzii* (a butyrate-producing bacteria), and increased *Ruminococcus gnavus* and Streptococcus spp (both are parts of the Firmicutes phylum) associated with the intake of NNS could indicate an obesogenic effect of NNS.

Conflicts of interest: None disclosed

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Key words: Gut microbiota; Non-nutritive sweeteners; Obesity; Short chain fatty acids;

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R&D AND BUSINESS COLLABORATIONS IN MICROBIOTA RESEARCH, PROBIOTICS, HEALTH AND DISEASE

20-22 May 2019, Rotterdam, The Netherlands

Invited speaker, Finn Terje Hegge

Title: Transition of microbiota analysis from research tool to clinical utility

The GA-map® technology represents a unique approach to microbiome analysis. By combining information from a well-defined set of pre-determined markers it enables highly reproducible and standardized information to be derived from the complex human gut microbiota. This information enables actionable results such as disease progression prediction and treatment response prediction. Examples of the clinical utility of the GA-map® technology will be presented.