Transition of microbiota analysis from research tool to clinical utility

6TH MICROBIOME R&D & BUSINESS COLLABORATION FORUM
Rotterdam, The Netherlands

Finn Terje Hegge, CTO

Genetic Analysis AS

- Genetic Analysis is a Norwegian diagnostic company focused on developing the GA-map® technology for mapping microbiota
- GA-map® is based on many years of research at the Norwegian University of Life Science by Prof. Knut Rudi and co-workers
- Currently 20 employees, and growing. In addition with a large network of key opinion leaders and consultants
- Products on the market in EU and US
- Complete in-house value chain, combined with strong global partnerships

BIO-RAD

Luminex®

BIOHIT HealthCare

Business model

Partners

- Sales of reagent kits to major laboratories
- Marketed through international partners, global and regional

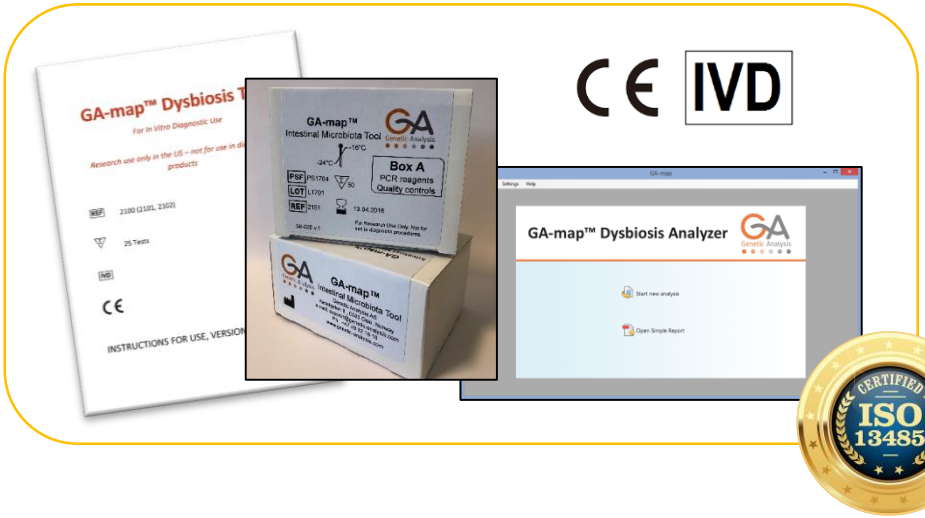
In house laboratory

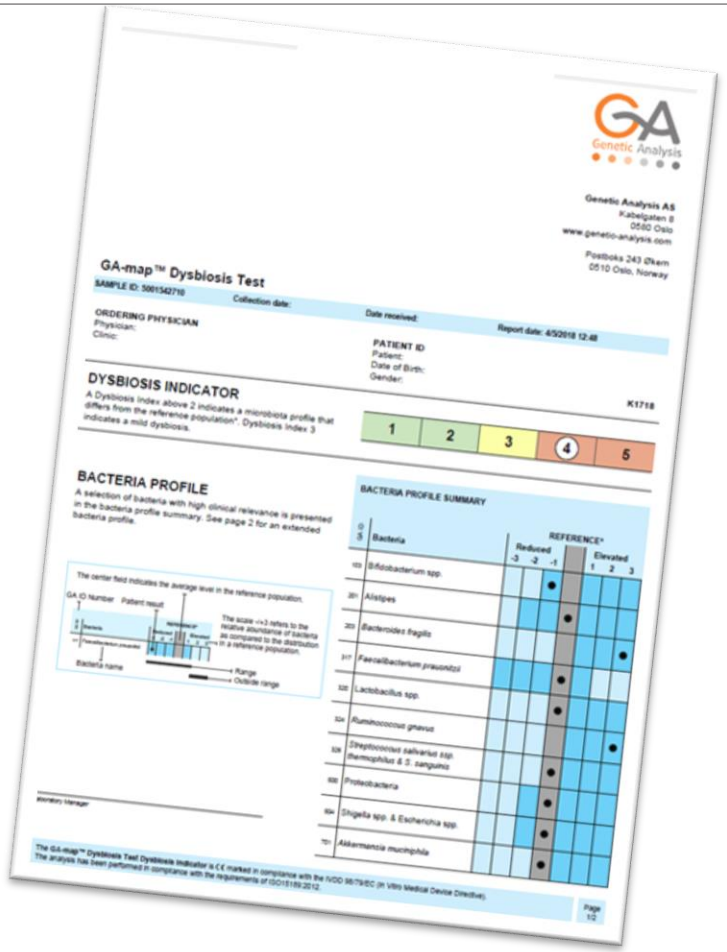
- Sales of lab services to the research and clinical market.
- Generating value through the use of our extensive database linking microbiota profiles to clinical outcomes

GA-map® Dysbiosis Test Lx

The GA-map® Dysbiosis Test Lx is intended to be used as

a gut microbiota DNA analysis tool to identify and characterize dysbiosis





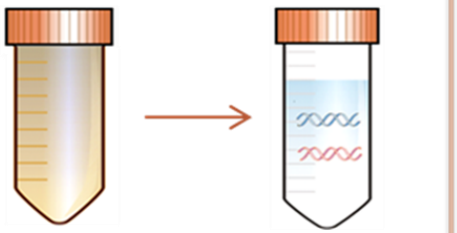
- A **standardized, documented and CE marked faecal IVD test** to easily profile the microbiome
- Simple to perform in any molecular laboratory
- Fast and easy to use and interpret

GA-map® platform

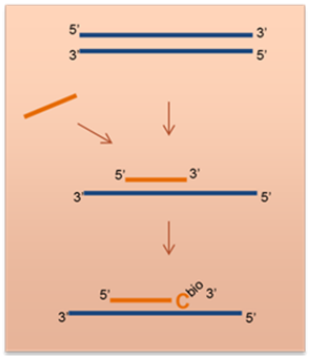
Reagent kit

- GA-map® Dysbiosis Test (CE-marked)
- ISO 13485 certified production facility

gDNA extraction



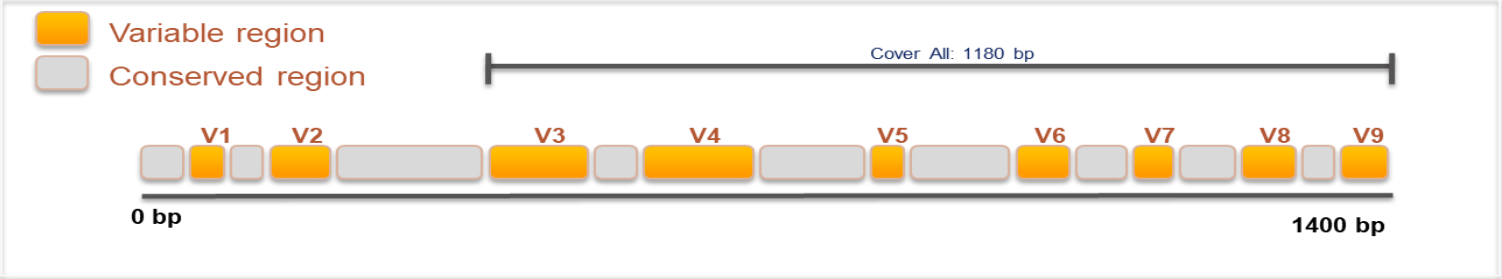
Probe labelling

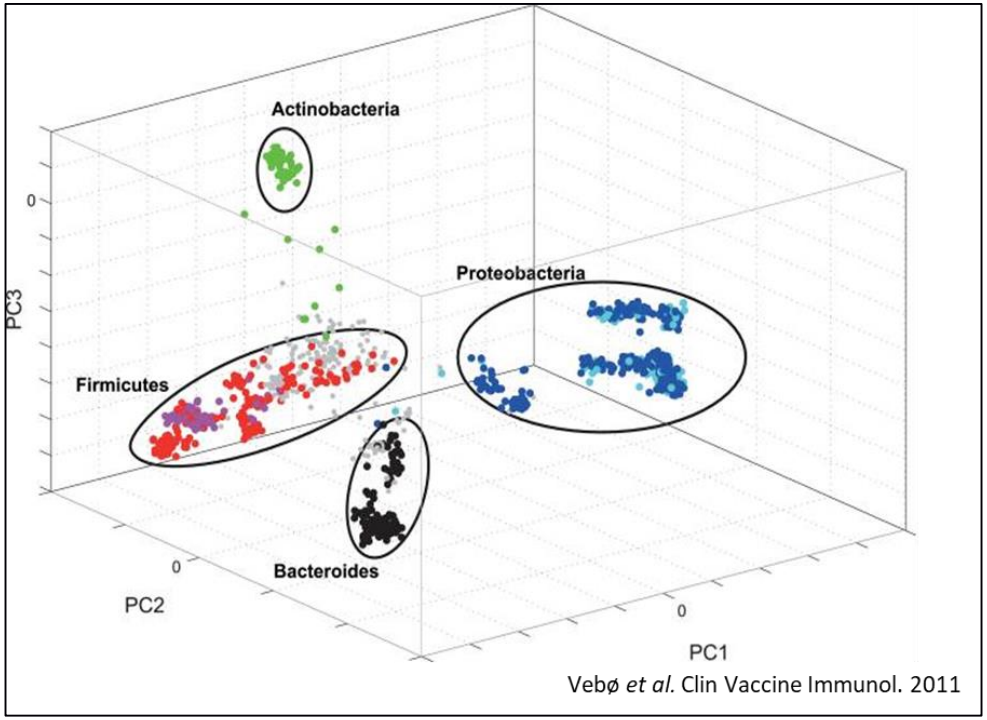


Detection

- Two peer-reviewed publications on methodology
- Several independent publications on use of method
- Six patent families

Defined set of pre-determined markers



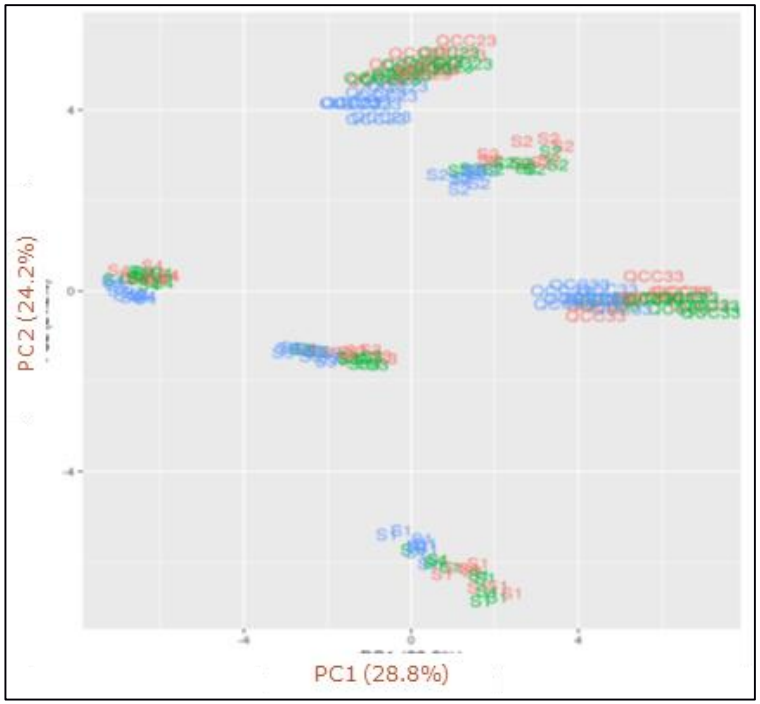


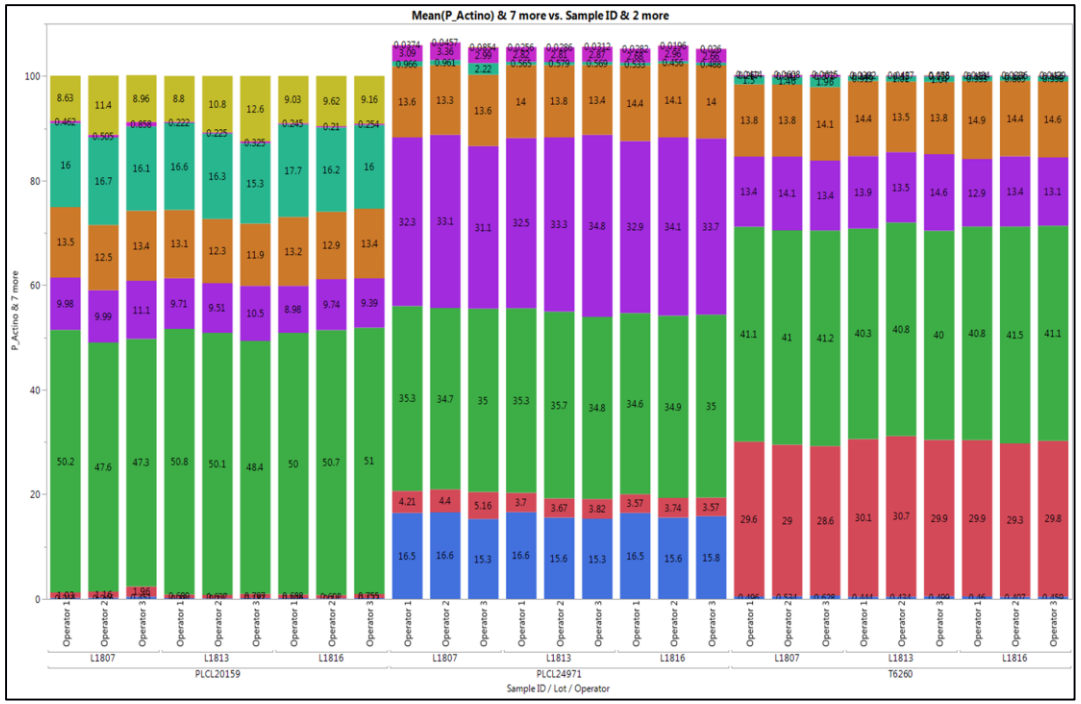
Example of 7 group probes represented by different colors. Each object represents one bacterial clone. The objects are clustered based on 16S rRNA gene phylogeny.

Two types of probes:

1) Specific probes – selected based on literature and KOLs feedback
2) Dysbiosis group probes – selected based on ability to separate normobiosis from dysbiosis

Technical performance





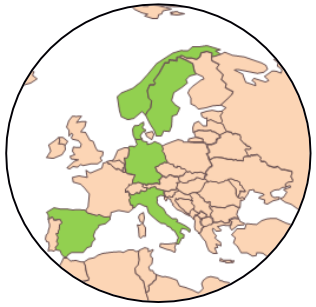
Parameter	Results	
Repeatability	2.6% CV	DI = 3.2 ± 0.04
Reproducibility	4.2% CV	DI = 3.2 ± 0.07

- Actinobacteria
- Bacteroidetes
- Firmicutes/Clostridia
- Firmicutes/Bacilli
- Firmicutes/Erysipelo-trichia & Negativicutes
- Proteobacteria
- Tenericutes
- Verrucomicrobia (*A. muciniphila*)

Based on a Normal Reference Range

Normobiotic reference based on approximately 300 samples from

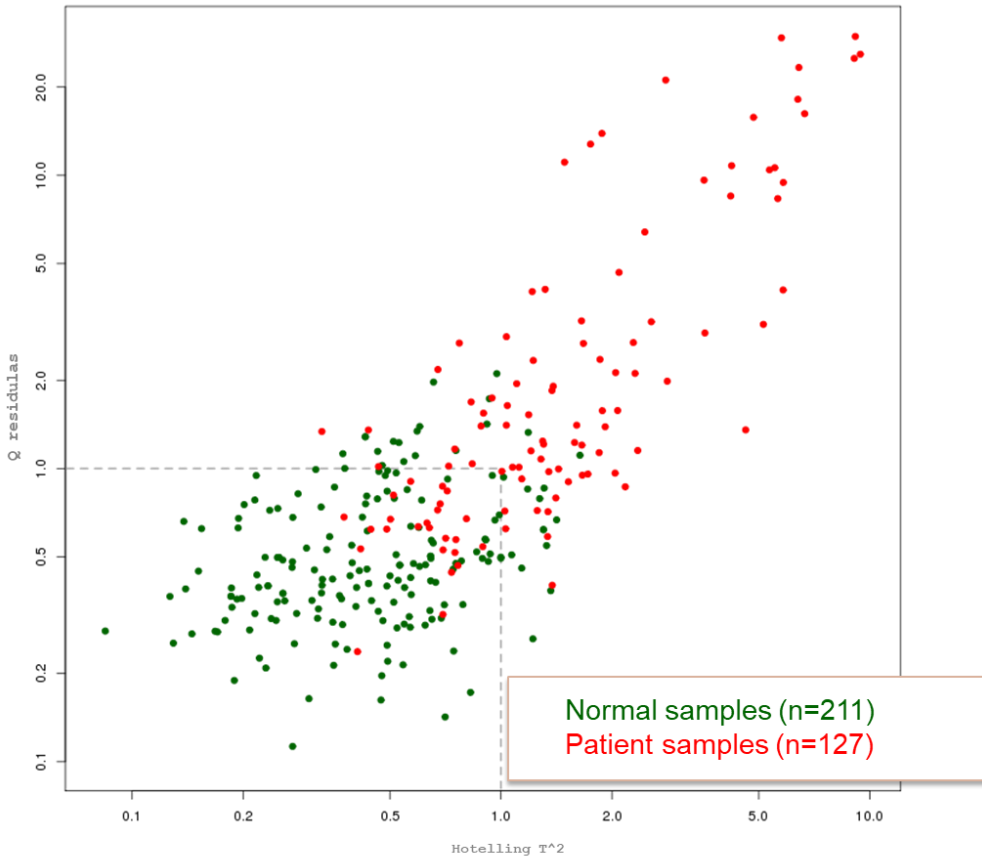
- Norway
- Sweden
- Denmark
- Germany
- Italy
- Spain



Clinical validation

Parameter	IBD	IBS
Dysbiotic	73%	69%
Normobiotic	27%	31%

GA-map® shows good separation of normal from diseased



- T2-Qres plot showing the cut-off line between a pre-defined normobiotic and dysbiotic state
- One point is one sample, and the points are coloured according to donor

Result output

Sample Physician
Sample Clinic
Long-name-of-some-street 123
Zip City

Genetic Analysis AS
Kabelgaten 8
0580 Oslo
www.genetic-analysis.com

Postboks 243 Økern
0510 Oslo, Norway

GA-map™ Dysbiosis Test

SAMPLE ID: SU0752 | Collection date: 2017-01-01 | Date received: 2017-01-02 | Report date: 21.06.2017 12:37

ORDERING PHYSICIAN
Physician: Sample Physician
Clinic: Sample Clinic

PATIENT ID
Patient: Firstname Lastname
Date of Birth: 1970-02-01
Gender: M

PS1408

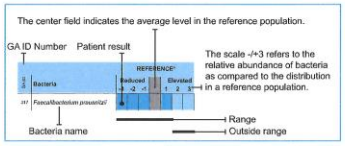
DYSBIOSIS INDICATOR

A Dysbiosis Index above 2 indicates a microbiota profile that differs from the reference population*. Dysbiosis Index 3 indicates a mild dysbiosis.

1	2	3	4	5

BACTERIA PROFILE

A selection of bacteria with high clinical relevance is presented in the bacteria profile summary. See page 2 for an extended bacteria profile.



BACTERIA PROFILE SUMMARY

GA ID	Bacteria	Reduced -3	-2	-1	REFERENCE*	Elevated 1	2	3
103	Bifidobacterium spp.				●			
201	Alistipes				●			
203	*Bacteroides fragilis*				●			
317	*Faecalibacterium prausnitzii*				●			
320	Lactobacillus spp.						●	
324	*Ruminococcus gnavus*					●		
326	*Streptococcus salivarius ssp. thermophilus & S. sanguinis*							●
500	Proteobacteria					●		
504	Shigella spp. & Escherichia spp.				●			
701	*Akkermansia muciniphila*				●			

Laboratory Manager

The GA-map™ Dysbiosis Test Dysbiosis Indicator is CE marked in compliance with the IVDD 98/79/EC (in Vitro Medical Device Directive).
The analysis has been performed in compliance with the requirements of ISO15189:2012.

BACTERIA PROFILE

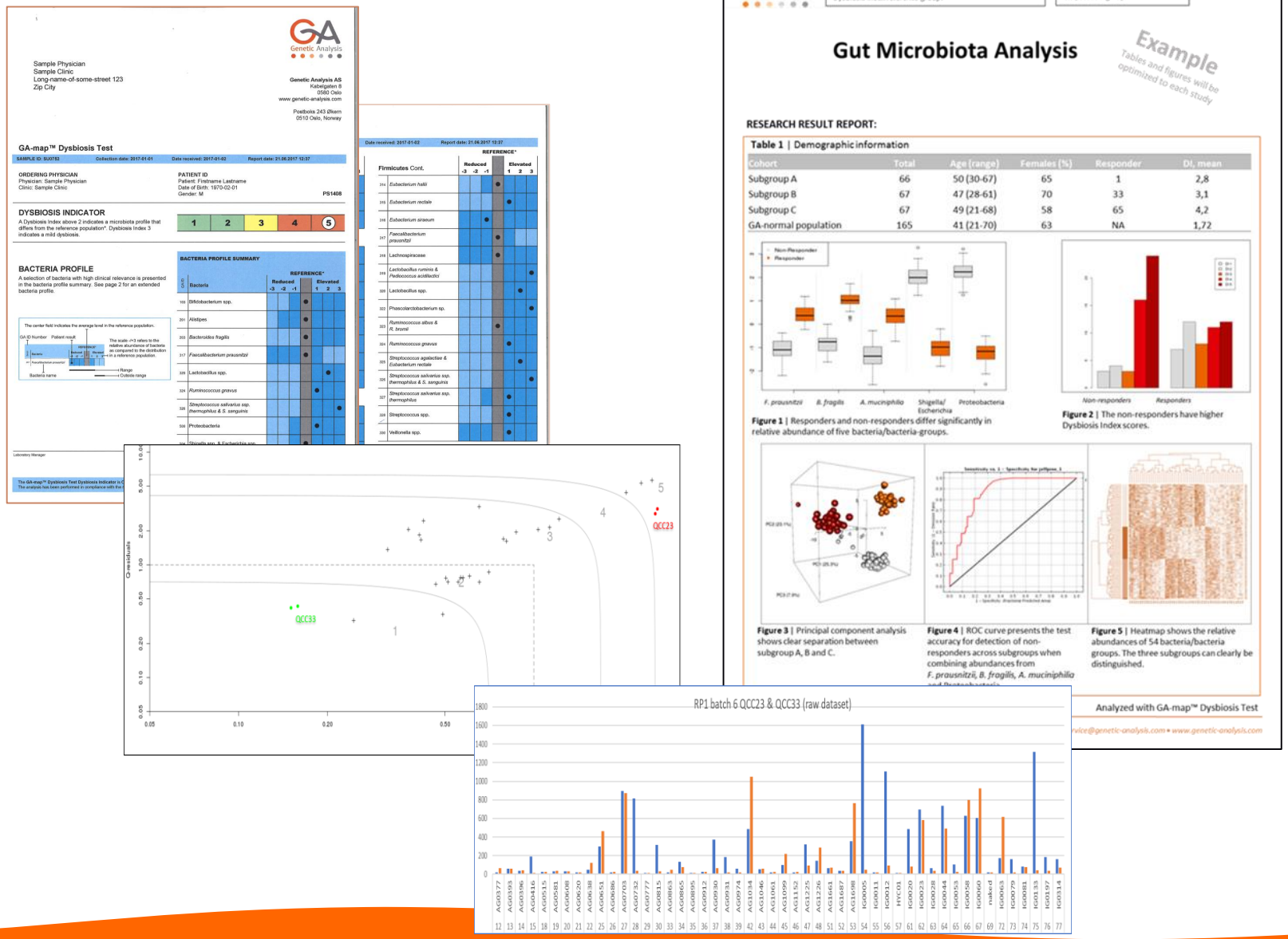
SAMPLE ID: SU0752 | Collection date: 2017-01-01 | Date received: 2017-01-02 | Report date: 21.06.2017 12:37

		Reduced -3	-2	-1	REFERENCE*	Elevated 1	2	3
Actinobacteria								
100	Actinobacteria				●			
101	Actinomycetales				●			
103	Bifidobacterium spp.				●			
Bacteroidetes								
201	Alistipes				●			
202	*Alistipes onderdonkii*				●			
203	*Bacteroides fragilis*				●			
206	Bacteroides spp. & Prevotella spp.				●			
207	*Bacteroides stercoris*					●		
208	*Bacteroides zoogleoformans*				●			
209	*Parabacteroides johnsonii*					●		
210	Parabacteroides spp.				●			
Firmicutes								
300	Firmicutes						●	
302	Bacilli					●		
304	*Catenibacterium mitsuokai*				●			
305	Clostridia				●			
307	Clostridium sp.				●			
310	*Dialister invisus*							●
311	*Dialister invisus & Megasphaera micronuciformis*						●	
312	Dorea spp.					●		
313	*Eubacterium biforme*				●			

		Reduced -3	-2	-1	REFERENCE*	Elevated 1	2	3
Firmicutes Cont.								
314	*Eubacterium hallii*				●			
315	*Eubacterium rectale*					●		
316	*Eubacterium siraeum*			●				
317	*Faecalibacterium prausnitzii*				●			
318	Lachnospiraceae				●			
319	*Lactobacillus ruminis & Pediococcus acidilactici*							●
320	Lactobacillus spp.						●	
322	Phascolarctobacterium sp.							●
323	*Ruminococcus albus & R. bromii*				●			
324	*Ruminococcus gnavus*					●		
325	*Streptococcus agalactiae & Eubacterium rectale*						●	
326	*Streptococcus salivarius ssp. thermophilus & S. sanguinis*							●
327	*Streptococcus salivarius ssp. thermophilus*					●		
328	Streptococcus spp.					●		
330	Veillonella spp.					●		
Proteobacteria								
500	Proteobacteria					●		
504	Shigella spp. & Escherichia spp.				●			
Tenericutes								
601	*Mycoplasma hominis*				●			
Verrucomicrobia								
701	*Akkermansia muciniphila*				●			

For more detailed information about the results, please contact the laboratory.
*Reference population: An unselected group of non-symptomatic individuals (age 18-70).

Result output – for clinical research



Strong scientific foundation

Extensive network

Oslo University Hospital

Karolinska Institutet

UNIKLINIK KÖLN

UNIVERSITY OF GOTHENBURG

Gemelli

Texas Children's Hospital

Haukeland University Hospital

UNIVERSITY OF COPENHAGEN

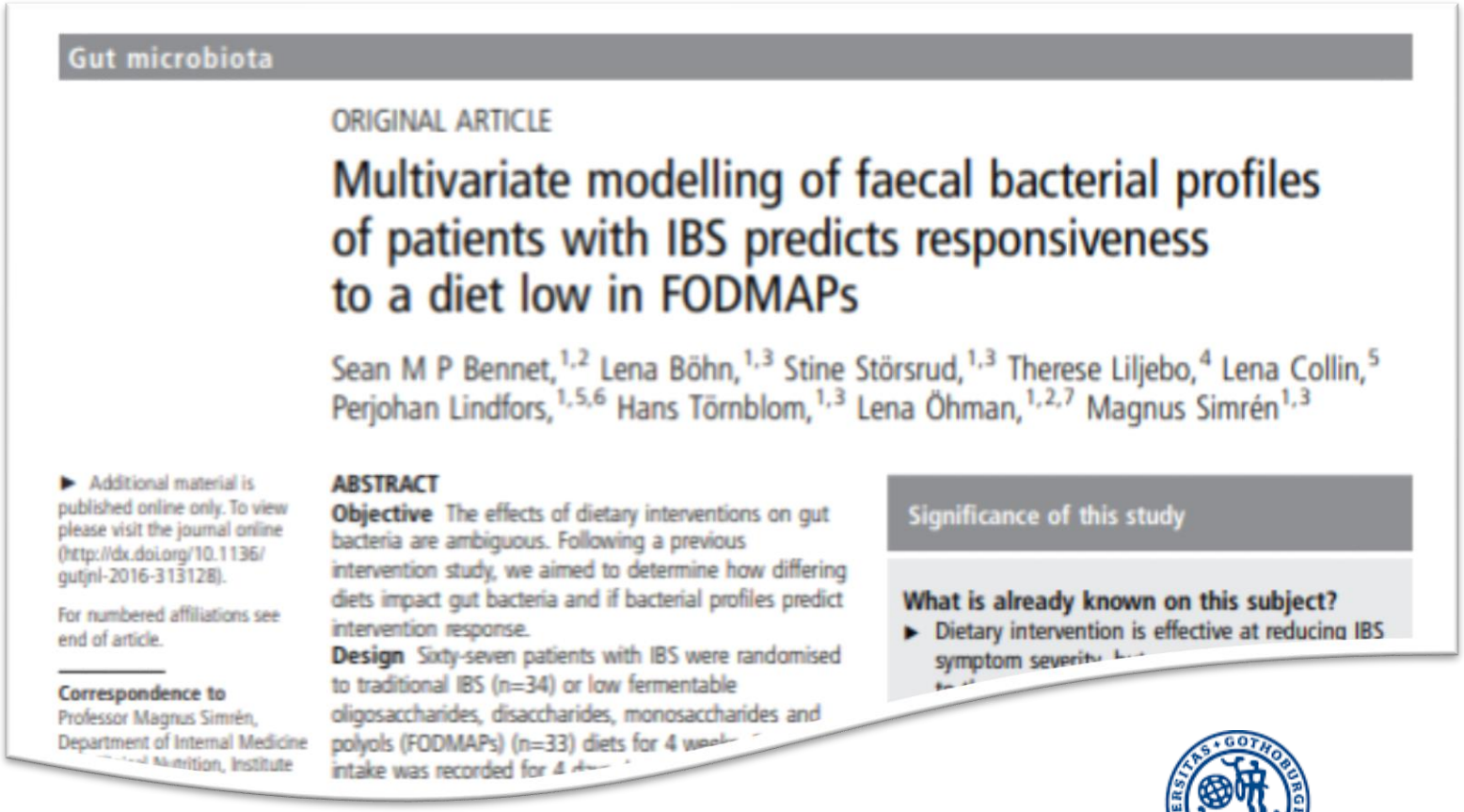
REGION Herlev Hospital

Azienda Ospedaliera Bolognini Seriate

University of Zurich[UZH]

Expanding scientific and clinical recognition

Prediction of responsiveness to low FODMAP diet



Gut microbiota

ORIGINAL ARTICLE

Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs

Sean M P Bennet,[1,2] Lena Böhn,[1,3] Stine Störsrud,[1,3] Therese Liljebo,[4] Lena Collin,[5] Perjohan Lindfors,[1,5,6] Hans Törnblom,[1,3] Lena Öhman,[1,2,7] Magnus Simrén[1,3]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2016-313128).

For numbered affiliations see end of article.

Correspondence to
Professor Magnus Simrén,
Department of Internal Medicine

ABSTRACT

Objective The effects of dietary interventions on gut bacteria are ambiguous. Following a previous intervention study, we aimed to determine how differing diets impact gut bacteria and if bacterial profiles predict intervention response.

Design Sixty-seven patients with IBS were randomised to traditional IBS (n=34) or low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) (n=33) diets for 4 weeks. Food intake was recorded for 4 days.

Significance of this study

What is already known on this subject?

► Dietary intervention is effective at reducing IBS symptom severity but

Motivation:

Not all IBS patients respond to the low FODMAP diet

Prediction of responsiveness to low FODMAP diet

Traditional IBS diet*

- Portion control and frequency. Eat three meals and three snacks during the day and to do so in a relaxed manner, chewing thoroughly and to a comfortable degree of fullness.
- Fibres were advised to be eaten distributed evenly over the day
- Reduce spicy and fatty foods, alcohol, coffee, onions, cabbage and beans.
- Avoid soft drinks, carbonated beverages and sweeteners that end with –ol (frequently found in chewing gums)

Low FODMAP diet*

- Restricted intake of foods containing FODMAPs.
- Avoid food rich in fructans and galactooligosaccharides (wheat, barley and rye, onion and certain legumes)
- Avoid lactose-containing products and foods with fructose in excess of glucose (apples, pears, asparagus, watermelon and honey)
- Avoid food items rich in mannitol, maltitol, sorbitol and xylitol (peaches, apricots and artificially sweetened products

**Böhn L, Störsrud S, Liljebo T, et al. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial. Gastroenterology 2015;149:1399–1407.*

Prediction of responsiveness to low FODMAP diet

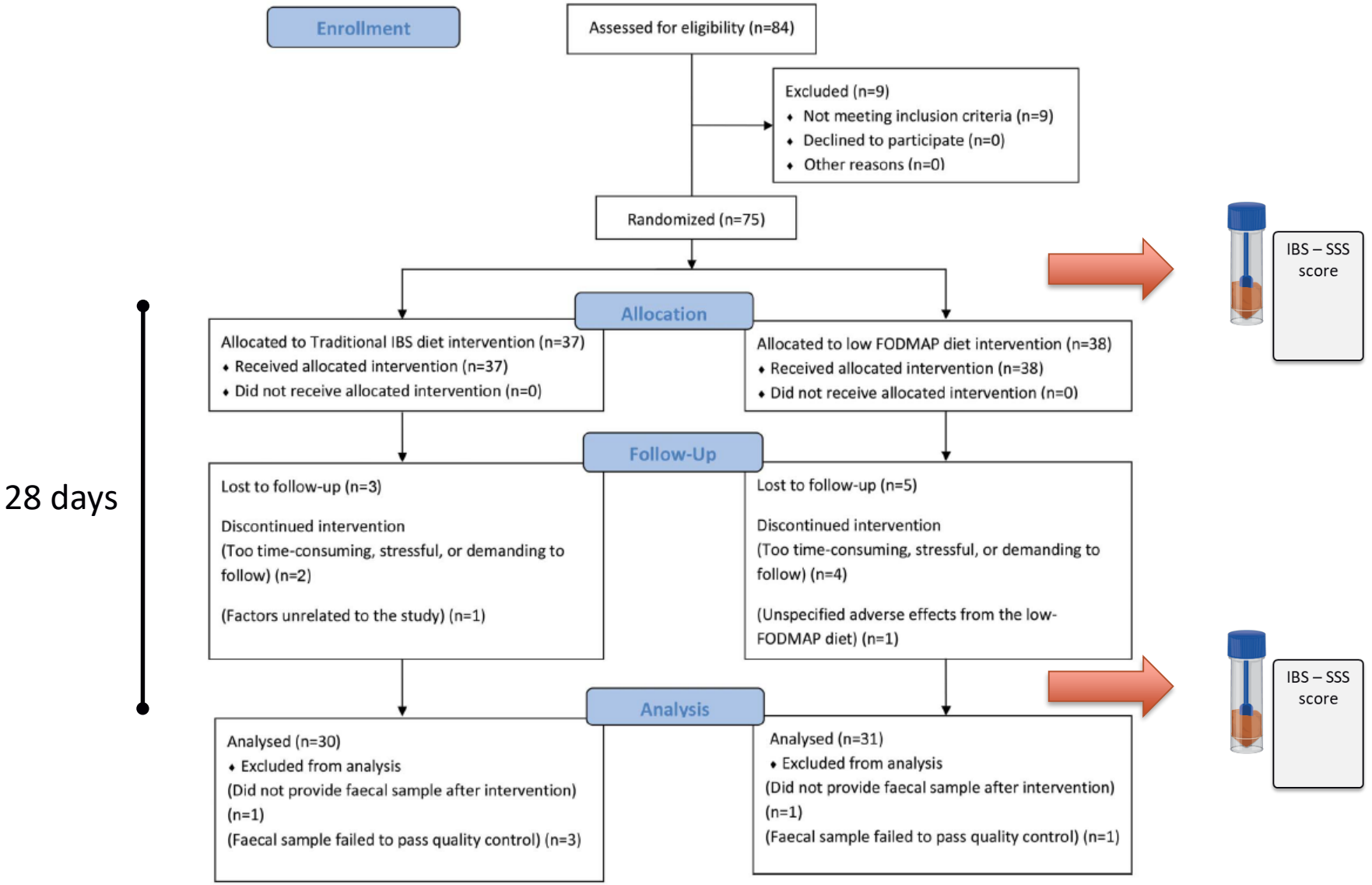
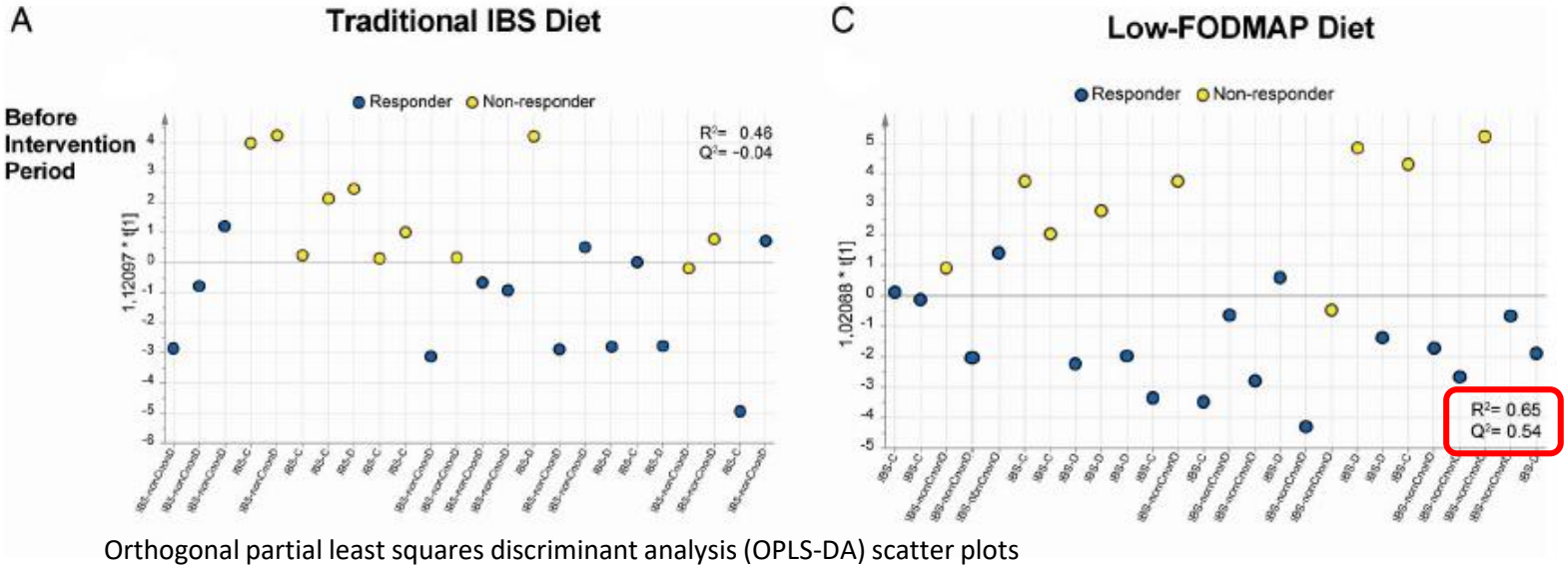


Figure 1 CONSORT flow diagram. Flow chart depicting patient numbers during the different phases of the study. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols.

Prediction of responsiveness to low FODMAP diet

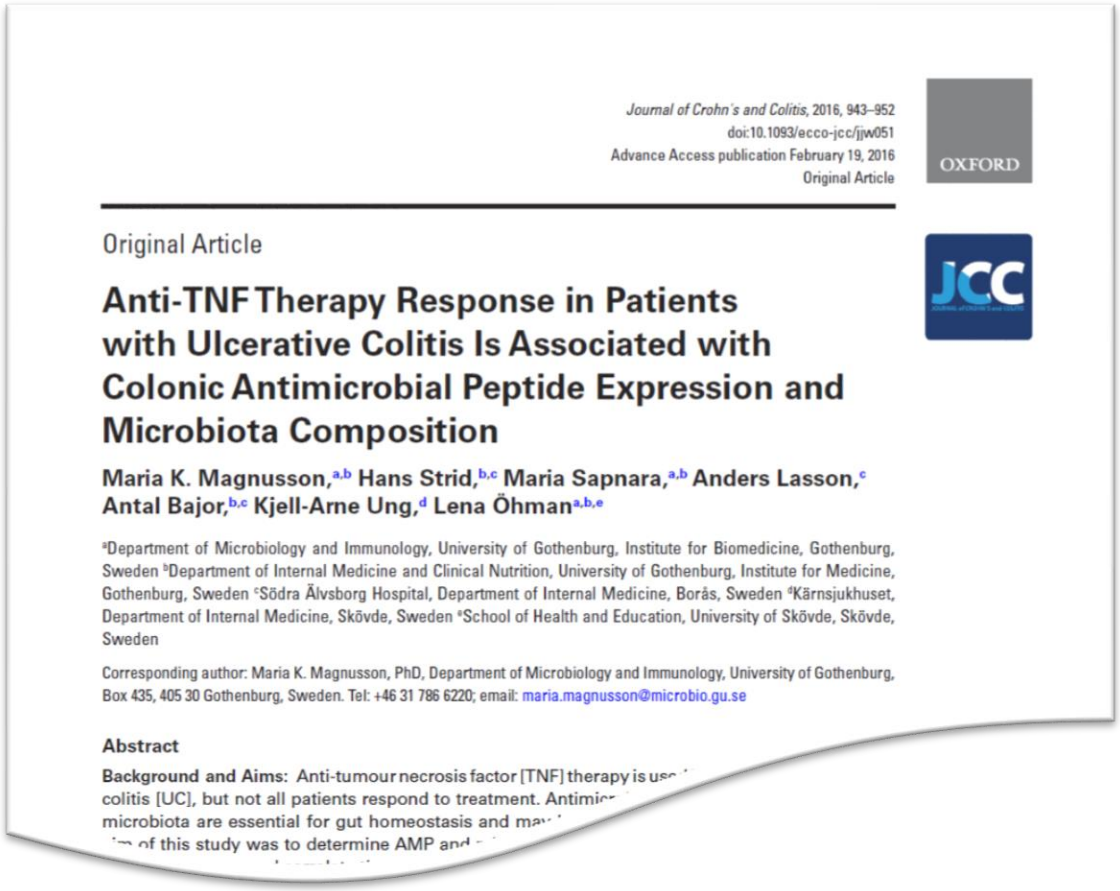


Orthogonal partial least squares discriminant analysis (OPLS-DA) scatter plots
R^2 – goodness of fit, R^2>0.5 is acceptable for biological data
Q^2 – predictive robustness, Q^2>0.4 is acceptable for biological data

Conclusion

Low FODMAP responders could be discriminated from non-responders before the intervention based on faecal bacterial profiles.

Anti-TNF Therapy response in UC patients



Journal of Crohn's and Colitis, 2016, 943–952
doi:10.1093/ecco-jcc/jjw051
Advance Access publication February 19, 2016
Original Article

OXFORD

JCC

Original Article

Anti-TNF Therapy Response in Patients with Ulcerative Colitis Is Associated with Colonic Antimicrobial Peptide Expression and Microbiota Composition

Maria K. Magnusson,[a,b] Hans Strid,[b,c] Maria Sapnara,[a,b] Anders Lasson,[c] Antal Bajor,[b,c] Kjell-Arne Ung,[d] Lena Öhman[a,b,e]

[a]Department of Microbiology and Immunology, University of Gothenburg, Institute for Biomedicine, Gothenburg, Sweden [b]Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Institute for Medicine, Gothenburg, Sweden [c]Södra Älvsborg Hospital, Department of Internal Medicine, Borås, Sweden [d]Kärnsjukhuset, Department of Internal Medicine, Skövde, Sweden [e]School of Health and Education, University of Skövde, Skövde, Sweden

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Abstract

Background and Aims: Anti-tumour necrosis factor [TNF] therapy is us
colitis [UC], but not all patients respond to treatment. Antimic
microbiota are essential for gut homeostasis and ma
of this study was to determine AMP and

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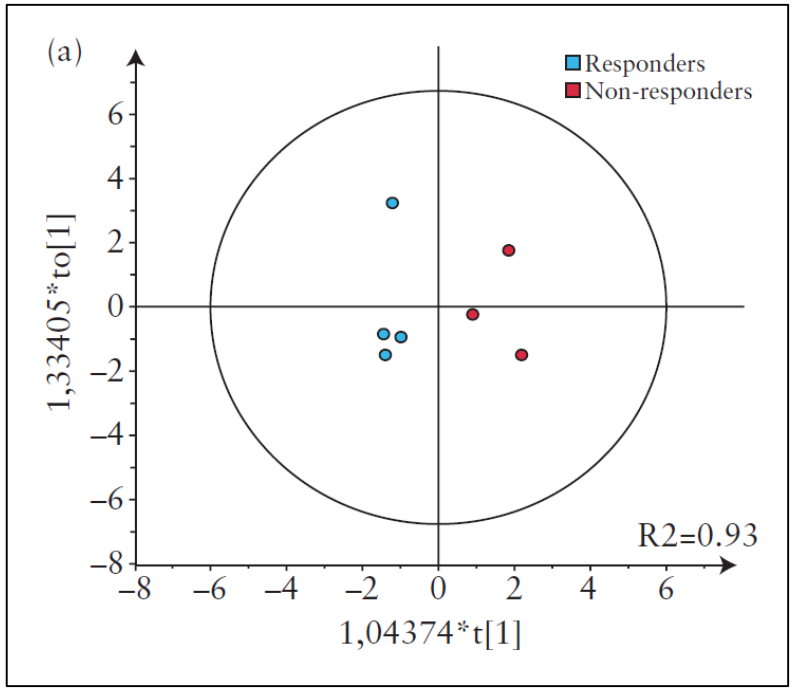
Motivation

Anti-tumour necrosis factor [TNF] therapy is used in patients with ulcerative colitis [UC], but not all patients respond to treatment

Anti-TNF Therapy response in UC patients



OPLS-DA of Anti-TNF therapy responders and non-responders differ at baseline with respect to their fecal microbiota composition. Fecal samples were obtained at baseline [responders *n* = 4 and non-responders *n* = 3] and analysed by the GA-map™ Dysbiosis Test. OPLS-DA scatter plot [A]

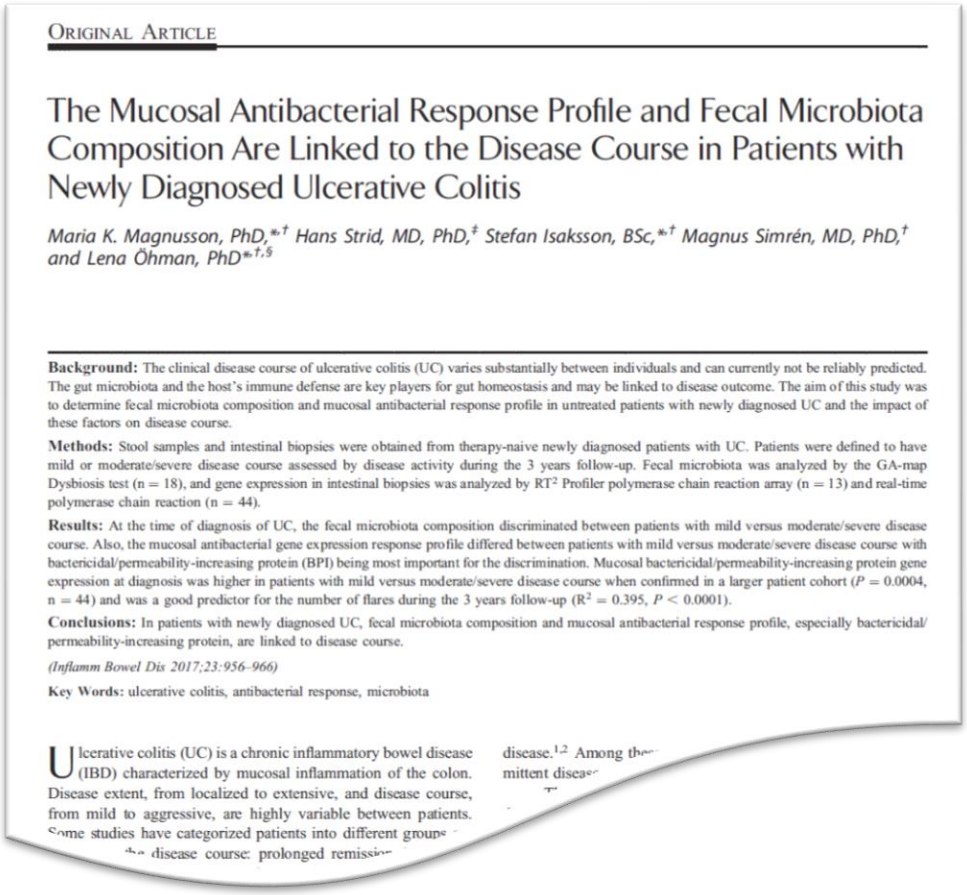
Stool samples for microbiota analysis were obtained from 7 patiens at baseline prior to 14 weeks intervention.

Treatment response was defined as a decrease in total Mayo score with ≥ 3 compared with baseline. Non-responders showed no improvement of the Mayo score.

Conclusion

Anti-TNF therapy responders and non-responders display distinctly separate gut microbiota profile before treatment start.

Disease course in IBD (UC) patients



ORIGINAL ARTICLE

The Mucosal Antibacterial Response Profile and Fecal Microbiota Composition Are Linked to the Disease Course in Patients with Newly Diagnosed Ulcerative Colitis

Maria K. Magnusson, PhD,† Hans Strid, MD, PhD,‡ Stefan Isaksson, BSc,*† Magnus Simrén, MD, PhD,† and Lena Öhman, PhD*†§*

Background: The clinical disease course of ulcerative colitis (UC) varies substantially between individuals and can currently not be reliably predicted. The gut microbiota and the host's immune defense are key players for gut homeostasis and may be linked to disease outcome. The aim of this study was to determine fecal microbiota composition and mucosal antibacterial response profile in untreated patients with newly diagnosed UC and the impact of these factors on disease course.

Methods: Stool samples and intestinal biopsies were obtained from therapy-naive newly diagnosed patients with UC. Patients were defined to have mild or moderate/severe disease course assessed by disease activity during the 3 years follow-up. Fecal microbiota was analyzed by the GA-map Dysbiosis test (n = 18), and gene expression in intestinal biopsies was analyzed by RT^2 Profiler polymerase chain reaction array (n = 13) and real-time polymerase chain reaction (n = 44).

Results: At the time of diagnosis of UC, the fecal microbiota composition discriminated between patients with mild versus moderate/severe disease course. Also, the mucosal antibacterial gene expression response profile differed between patients with mild versus moderate/severe disease course with bactericidal/permeability-increasing protein (BPI) being most important for the discrimination. Mucosal bactericidal/permeability-increasing protein gene expression at diagnosis was higher in patients with mild versus moderate/severe disease course when confirmed in a larger patient cohort ($P = 0.0004$, n = 44) and was a good predictor for the number of flares during the 3 years follow-up ($R^2 = 0.395$, $P < 0.0001$).

Conclusions: In patients with newly diagnosed UC, fecal microbiota composition and mucosal antibacterial response profile, especially bactericidal/permeability-increasing protein, are linked to disease course.

(Inflamm Bowel Dis 2017;23:956–966)

Key Words: ulcerative colitis, antibacterial response, microbiota

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by mucosal inflammation of the colon. Disease extent, from localized to extensive, and disease course, from mild to aggressive, are highly variable between patients. Some studies have categorized patients into different groups ... the disease course: prolonged remissi... disease.[1,2] Among th... mittent disea...

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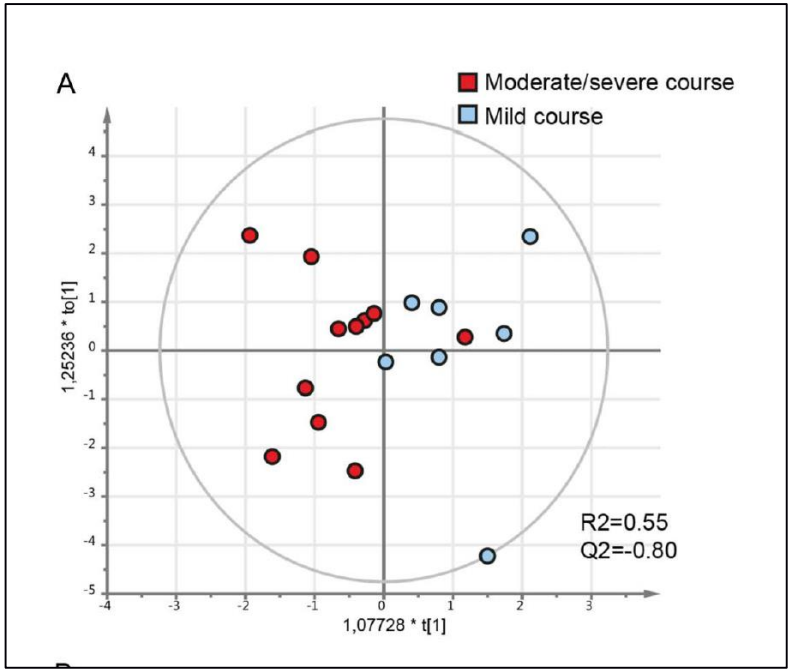
THE SAHLGRENSKA ACADEMY

Motivation

The clinical disease course of ulcerative colitis (UC) varies substantially between individuals and can currently not be reliably predicted.

Disease course in IBD (UC) patients



OPLS-DA of patients with mild (n=11) or moderate/severe (n=7) disease course differ at baseline with respect to their fecal microbiota composition.

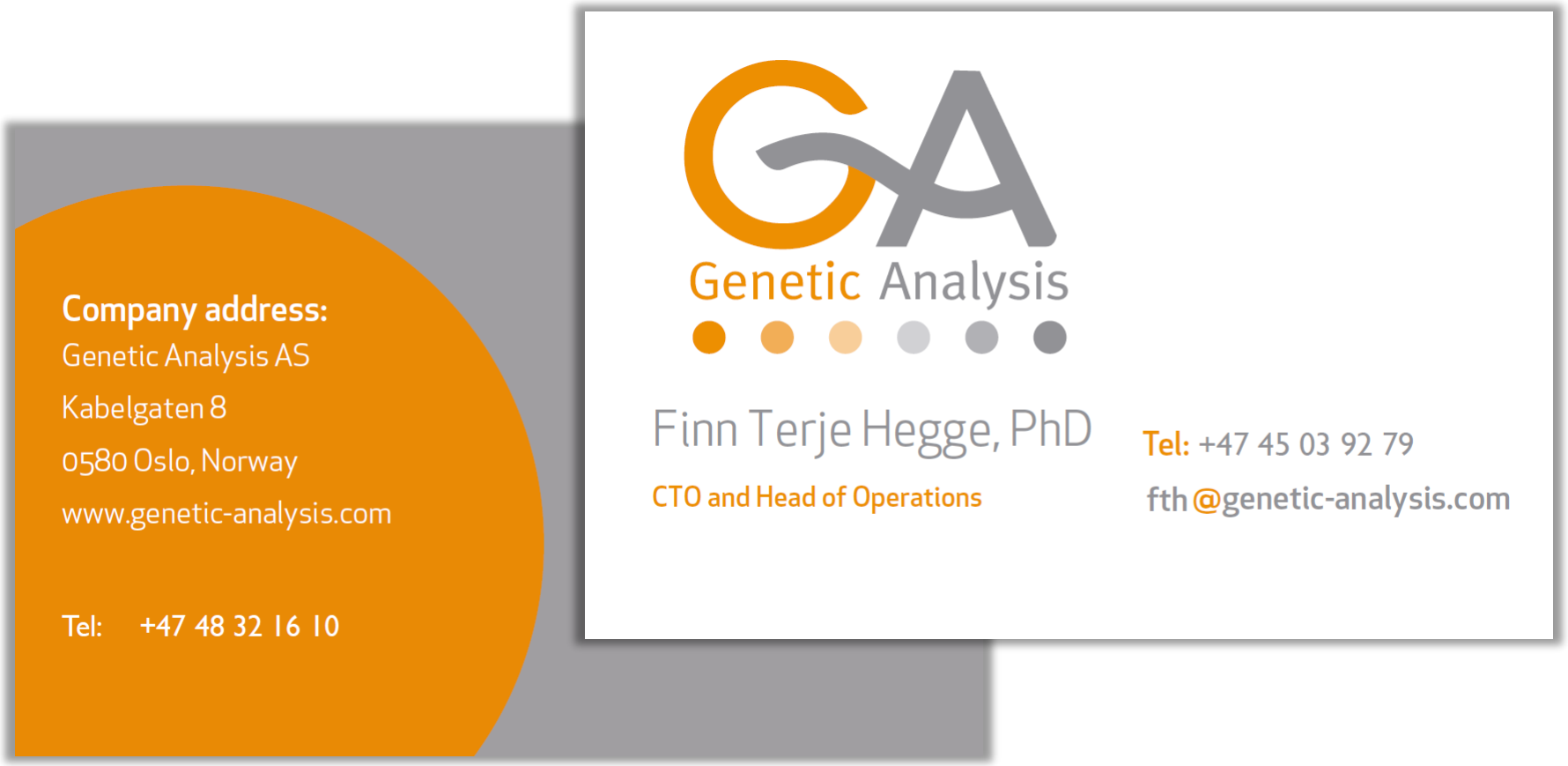
Stool samples were obtained from 18 therapy-naive newly diagnosed patients with UC.

Patients were defined to have mild or moderate/severe disease course assessed by disease activity during the 3 years follow-up.

Conclusion:

In patients with newly diagnosed UC, fecal microbiota composition are linked to disease course.

Thank you!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