10th National Microbiota Conference

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Radisson Blu Scandinavia Hotel

UiO : Institute of Clinical Medicine
University of Oslo

Oslo University Hospital

UiO : Life Science
University of Oslo

NO PSC
Norwegian PSC Research Center

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Abstracts

Metagenome sequencing of the gut microbiome reveal regional differences within Norway

Fjørtoft JHMS[1], Bøhn SK[2], Kværner AS[3], Berstad P[4], Rounge TB[3,4]

[1]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway; [2]Section for Colorectal Cancer Screening, Cancer Registry of Norway, Oslo, Norway; [3]Centre for Bioinformatics, Department of Pharmacy, University of Oslo, Norway; [4]Department of Research, Cancer Registry of Norway, Oslo, Norway.

The human gut microbiome exhibits remarkable diversity across geographical regions, suggesting a potential link between geographical residency and composition of the gut microbiome. Regional residency, along with variations in diet, lifestyle and socioeconomic status, may contribute to the observed differences in the gut microbiome among individuals.

Here, we explored regional differences in the gut. Using shotgun metagenomes from a cohort of 1036 colorectal cancer screening participants, we estimated α- and β-diversity and performed differential abundance and dietary correlation analyses on participants residing in two distinct geographical regions in South-East Norway (Moss and Bærum region) and centrality classes (class 1, 2 and $\geq$3). Overall, there were no regional differences in α-diversity between the regions and centrality classes, but small significant differences in β-diversity were observed. Analysis of differential abundance revealed enrichment of beneficial species associated with healthier diet and lifestyle, and higher socioeconomic status, such as *Eubacterium hallii*, *Faecalibacterium prausnitzii*, *Fusicatenibacter saccharivorans* and *Gemmiger formicilis*, in participants in the Bærum region and in those who lived centrally. Conversely, these participants displayed depletion in detrimental species, including *Escherichia coli*, *Eggerthella lenta*, *Flavonifractor plautii* and *Ruminococcus gnavus*, which were correlated with unhealthy lifestyle and lower socioeconomic status. These findings reveal a small but significant association between de-central residency and an unfavorable microbiome profile. Moreover, a healthier diet and lifestyle pattern, as well as higher socioeconomic status, were linked to a beneficial microbiome profile. Our study shows that within a homogenous study population, distinct geographic regions can foster variations in the gut microbiome.

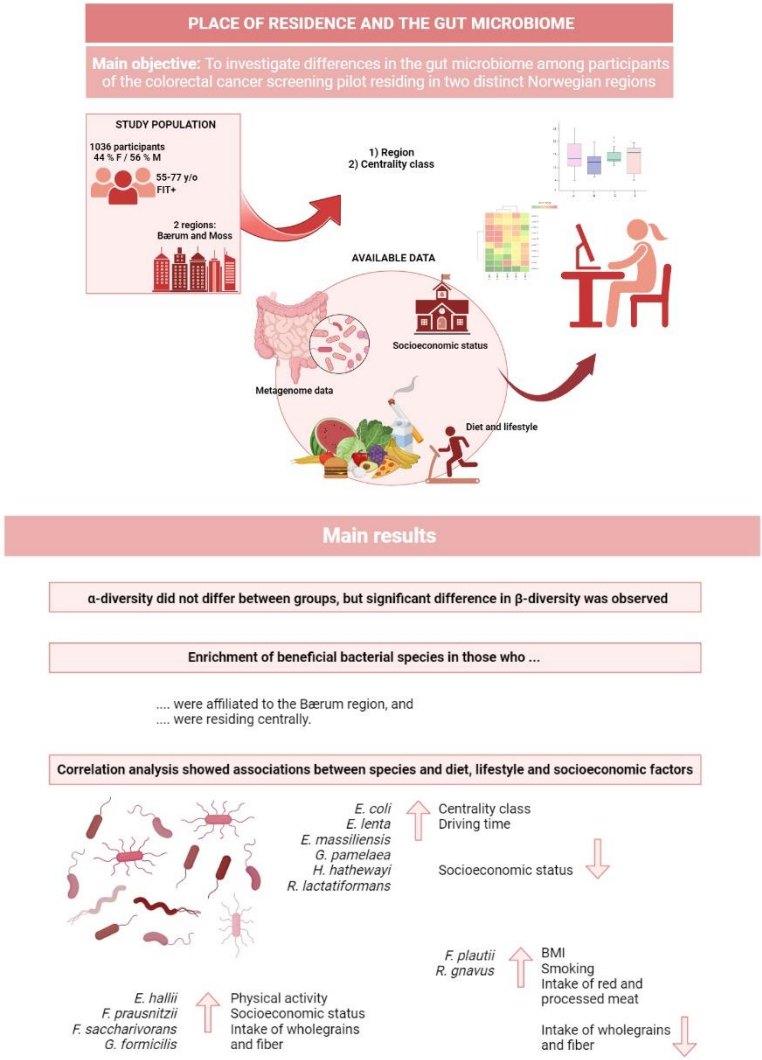

Fig. 1: Graphical abstract

Intestinal fatty acid binding protein and myocardial infarction sequelae in STEMI patients with acute heart failure

Nendl A[1,2], Andersen GØ[3], Husebye T[3], Trøseid M[1,4], Seljeflot I[1,2], Awoyemi A[3]

[1]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [2]Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway; [3]Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway; [4]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway.

Background: Reduced cardiac output, vasoconstriction, and congestion in acute heart failure (AHF) may damage the intestinal mucosa and disrupt its barrier function. This could facilitate leakage of bacterial products into circulation and contribute to the systemic inflammation and adverse cardiac remodeling frequently observed in these patients. We aimed to investigate the relationship between intestinal fatty acid binding protein (I-FABP) and myocardial infarction (MI) sequelae after 6 weeks in patients with ST-elevation MI (STEMI) complicated with AHF.

Methods: We enrolled 61 STEMI patients who developed AHF within 48 hours of successful revascularization by percutaneous coronary intervention (PCI). Serial blood samples were taken to measure levels of I-FABP as a marker of gut epithelial damage. I-FABP burden was estimated by calculating area under the curve from baseline to day 5. Serial echocardiography was performed to assess left ventricular ejection fraction (LVEF), global longitudinal strain (GLS) and wall motion score index (WMSI). Single photon emission computed tomography (SPECT) was performed at 6 weeks to determine infarct size and LVEF.

Results: At 6 weeks, I-FABP$_{AUC}$ correlated positively with infarct size measured by SPECT (rho=0.45, p=0.002), GLS (rho=0.32, p=0.03) and WMSI (rho=0.45, p=0.001), and negatively with LVEF measured by SPECT (rho=-0.40, p=0.007) and by echocardiography (rho=-0.33, p=0.02). At 6 weeks, I-FABP$_{AUC}$ above median predicted LVEF measured by SPECT and by echocardiography below median, and GLS and WMSI above median (see table).

Conclusion: I-FABP, a marker of gut epithelial damage, may predict the extent of myocardial damage and cardiac function in primary PCI-treated STEMI patients with *de novo* AHF.

Table:

	p-value	Odds ratio	95% CI for odds ratio	
			Lower	Upper
LVEF by SPECT below median	**0.02**	5.62	1.33	23.7
LVEF by echo below median	**0.04**	4.06	1.04	15.86
GLS above median	**0.01**	10.76	1.72	67.43
WMSI above median	**0.02**	6.36	1.33	30.48
Infarct size above median	0.24	2.24	0.58	8.64

Gut dysbiosis and neutrophil extracellular traps (NETs) in heart failure

Bratseth V[1], Nendl A[1,2], Seljeflot I[1,2], Raju SC[2,3], Trøseid M[2,4], Awoyemi A[1,5]

[1]Oslo Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway; [2]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [3]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; [4]Section of Clinical Immunology, and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway; [5]Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway.

Background: An increasing body of evidence links heart failure (HF) to gut dysbiosis. Low bacterial diversity, and an increase in harmful microbial metabolites such as trimethylamine N-oxide (TMAO), have previously been reported. Dysbiosis may also lead to gut barrier dysfunction and microbial translocation that may in turn activate neutrophils with release of neutrophil extracellular traps (NETs) (NETosis). We aimed to assess associations between gut dysbiosis, cardiac function and blood biomarkers of NETs, largely unexplored in chronic HF.

Methods: We included patients with chronic HF and left ventricular ejection fraction (LVEF) <40% (n=151). The microbial dysbiosis-index (DI) was calculated for each sample, based on differentially abundant bacterial species from the MaAsLin2-analysis. Shannon diversity-index was used as a measure of microbiota diversity. Severe HF was defined as N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations above median, and LVEF below median (31%). Markers of NETosis included citrullinated-histone-H_3 (H_3Cit), double-stranded DNA (dsDNA) and myeloperoxidase (MPO)-DNA.

Results: In HF patients, DI and TMAO were positively correlated with dsDNA (r=0.221, p=0.012 and r=0.168, p=0.043, respectively. Shannon diversity-index was inversely correlated with H_3Cit (r=-0.220, p=0.012). Patients with severe HF (NT-proBNP>895 pg/ml) had significantly higher concentrations of H_3Cit (p=0.013), and those with LVEF below median had higher concentrations of H_3Cit (Q4)(p=0.025).

Conclusion: In patients with chronic HF, gut microbiota dysbiosis and TMAO associated with dsDNA. The NETs marker H_3Cit was associated with HF severity and low microbiota diversity. We hypothesize that gut dysbiosis in chronic HF contributes to activation of innate immunity through NETosis as part of disease development.

Oral microbiota in carotid atherosclerosis

Stø K[1,2], Skagen KR[2], Holm K[1,3,4], Vestad B[3,4], Bjerkeli V[1], Trøseid M[1,3,5], Hov JR[1,3,4,6], Skjelland M[1,2]

[1]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [2]Department of Neurology, Oslo University Hospital, Oslo, Norway; [3]Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway; [4]Section of Gastroenterology, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway; [5]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Oslo, Norway; [6]Norwegian PSC Research Center, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway.

Background: Ischemic stroke is a frequent cause of mortality and disability worldwide. Carotid atherosclerosis is the cause of around 20% of these strokes. We aimed to investigate the association between oral microbiota, carotid atherosclerosis and risk factors for ischemic stroke.

Methods: Patients with severe carotid atherosclerosis (i.e. ≥50% stenosis) (n=60) were compared with healthy controls (n=44). Carotid arteries were investigated with ultrasound. Unprovoked saliva and oral swab samples (i.e. dry cotton bud swab of inner and outer surfaces of upper and lower teeth rows and tongue) were collected in fasting participants. Bacterial DNA was extracted from material types from all participant, and oral microbiota composition was analyzed by 16S rRNA amplicon sequencing targeting the V3-V4 region and the QIIME2 bioinformatic pipeline. ANCOM-BC2 was used for abundance comparisons, with a false discovery rate threshold of <0.2.

Results: Following data processing and QC, 145 samples from 78 individuals were included in the final analysis comprising 6,396,158 reads, with a minimum read count of 7,086 per sample. The overall microbiota composition in saliva and oral swabs were distinct but closely correlated (rho 0.71, p=0.001). Considering intra-individual (alpha) diversity, this was reduced as measured by numbed of observed features in people with severe carotid atherosclerosis compared with healthy controls (p=0.008 and p=0.02 in saliva and oral swabs, respectively). Considering the bacterial composition analysis, 35 and 43 genera were altered in patients vs. controls considering saliva and oral swabs, respectively. A lower alpha diversity correlated with increased leukocyte count, statin use and antiplatelet use, as well as increased degree of carotid stenosis.

In a subsequent a comparison of people with symptomatic (i.e. ischemic stroke or TIA within the last 3 months) and asymptomatic carotid atherosclerosis, alpha diversity was found reduced at similar levels in the two groups. In contrast, several bacterial genera were different in symptomatic individuals in both saliva and oral swabs, including an increase in *Eikenella*, which has previously been associated with atherosclerosis in humans.

Conclusion: Oral microbiota composition differs between patients with carotid atherosclerosis and healthy controls in terms of different genera abundances, and lower alpha diversity in the patient group.

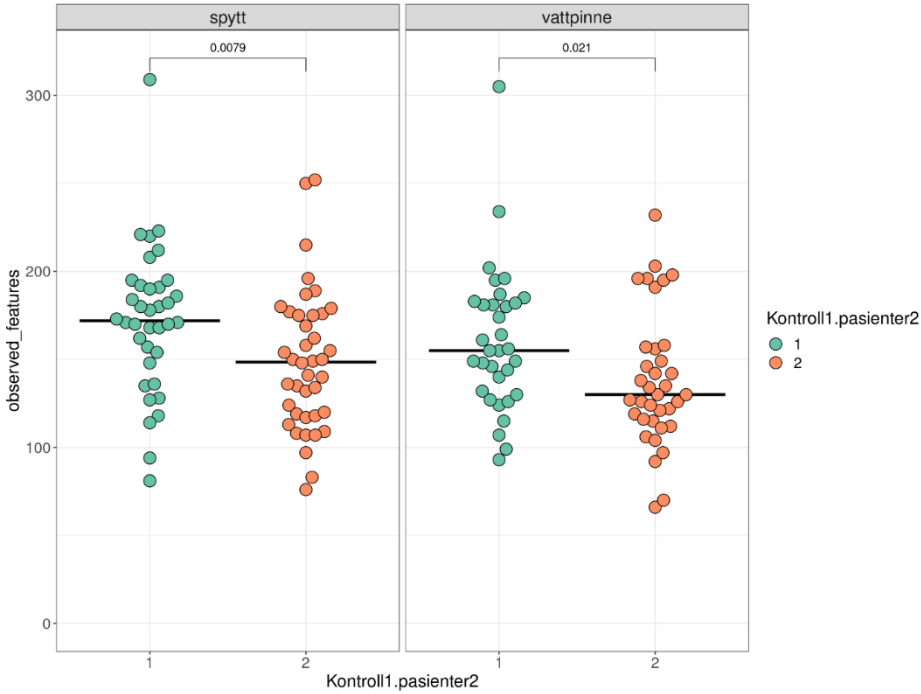

Fig. 1: Observed features

Microbial-derived imidazole propionate links the heart failure-associated microbiome alterations to disease severity

Raju SC*[1,2], Molinaro A*[1,3,4], Awoyemi A[5,6], Jørgensen SF[1,7], Braadland PR[2,4], Nendl A[5,6], Seljeflot I[5,6], Ueland PM[8], McCann A[8], Aukrust P[1,2,7], Vestad B[1,4], Mayerhofer C[1], Broch K[9], Gullestad L[2,9], Lappegård KT[10,11], Halvorsen B[1,2], Kristiansen K[12,13], Hov JR[#1,4,14], Trøseid M[#1,2,7]

[1]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; [2]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway; [3]Department of Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden; [4]Norwegian PSC Research Center, Department of Transplantation Medicine, Oslo University Hospital Rikshospitalet, Norway; [5]Department of cardiology, Oslo University Hospital Ullevål, Oslo, Norway; [6]Center for clinical heart research, Oslo University Hospital Ullevål, Oslo, Norway; [7]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway; [8]Bevital, Bergen, Norway; [9]Department of cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway. [10]Division of Internal Medicine, Nordlandssykehuset, Bodø, Norway; [11]Institute of Clinical Medicine, University of Tromsø, 9037 Tromsø, Norway; [12]BGI-Shenzhen, Shenzhen, China; [13]Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark; [14]Section of Gastroenterology, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway.
**Shared first authorship*
 # Shared senior authorship

Interactions between the gut microbiota, diet, and host metabolism contribute to the development of cardiovascular disease, but a firm link between disease-specific gut microbiota alterations and circulating metabolites is lacking. Here, we show that heart failure (HF) displays a specific compositional and functional shift of the gut microbiota that is linked to circulating levels of the microbial histidine-derived metabolite imidazole propionate (ImP).

Circulating levels of ImP are elevated in HF and associate with a dysbiotic, pro-inflammatory microbiota as well as indices of systemic inflammation. Contrary to the microbiota composition, circulating levels of ImP provide insights into HF etiology and severity indices, demonstrating a direct link between HF-related dysbiosis and host health not otherwise evident due to the heterogeneous gut microbial composition observed in subjects with HF.

The Liver Filter - Using liver transplantation as a filtering tool to identify gut microbial signals in primary sclerosing cholangitis (PSC)

Braadland PR[1], Hradicka P[1], Molinaro A[1], Engesæter LK[1], Lin W[2], Frank A[1], Ulvik A[3], Ueland PM[3], Melum E[1], Line PD[4], Karlsen TH[1], Globisch D[2], Hov JR[1]

[1]Norwegian PSC Research Center, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway; [2]Department of Chemistry BMC, Science for Life Laboratory, Uppsala University, Uppsala Sweden; [3]BEVITAL AS, Bergen, Norway; [4]Institute of Clinical Medicine, University of Oslo, Oslo, Norway.

The gut microbiome is a major determinant of the human blood metabolome, and microbial metabolites have been linked to several diseases. The liver sits at the interaction between the host and the gut where it is exposed to microbial metabolites transported via portal blood.

PSC is a prototypical disease of this gut-liver axis, characterized by gut dysbiosis, concomitant inflammatory bowel disease and liver failure. PSC commonly recurs in liver transplant (LT) recipients despite considerable immunosuppression. The gut dysbiosis does not resolve after LT for PSC, overall suggesting that gut microbial signals act as disease drivers across the natural history of PSC.

Using untargeted metabolomics, we find that people with PSC have a notably altered circulating metabolome. LT largely reverts these changes, indicating that most metabolomic changes are consequences of worsening liver disease. Although this makes it challenging to pinpoint bacterial metabolites, it represents an opportunity, since metabolites *not* changed after LT are enriched for bacterial metabolites, some of which we find to be potential disease modifiers.

We demonstrate the feasibility of using LT as a filtering to identify gut bacterial metabolites. In the next step, we will use novel LC-MS/MS-based technology to profile metabolites typically produced by gut bacteria in cross-sectional and longitudinal PSC cohorts. With the aim to identify actionable targets for PSC, for which no such drug-based therapies exist, we will test these metabolites' associations with clinical outcomes and their effects in preclinical models.

Human microbiota associated mouse models: tool to study host-microbe interactions

Hradicka P[1,2,3], Rasmussen H[3,4], Braadland PR[1,2,3], Holm K[1,2,3], Schneditz G[1,2,3], Bjørnholt JV[3,5], Melum E[1,2,3,6,7], Hov JR[1,2,3,6]

[1]Norwegian PSC Research Center, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [2]Research institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [3]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [4]Department of Comparative Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [5]Department of Microbiology, Oslo University Hospital, Oslo, Norway; [6]Section of Gastroenterology, Department of transplantation medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [7]Hybrid Technology Hub-Centre of Excellence, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway.

Introduction: The complex interplay between gut microbes and host physiology can be experimentally studied by transplanting human-derived microbial communities into mice. The primary objective of this study was to conduct an in-depth examination of the microbiota and metabolome in human microbiota-associated mice, with a specific focus on liver cholestatic disease.

Material and Methods: In the present study, germ-free C57BL/6J mice (males and females) were used and divided into 3 groups: germ-free group (n=4), healthy control (HC) group colonized by fecal microbiota transplant (FMT) from human HC (n=30) and primary sclerosing cholangitis (PSC) group colonized by FMT from patients with PSC (n=35). All animals were subjected to DDC treatment to induce cholestatic liver disease. At the end of the study, plasma samples for metabolomics, and fecal and mucosal samples for 16S rRNA sequencing were collected (Fig. 1).

Results: PSC and HC donors did not show any difference in the intra-individual (alpha) diversity (n=7 in both groups), while the diversity was reduced in recipient's mice suggesting loss of microbes during transplantation. The gut microbiota of "PSC mice" was different and less diverse than "HC mice". 80% of the differences in microbiota composition could be explained by the donor, whereas the composition did not associate with the severity of cholestatic disease in the mice.

Plasma metabolomics was performed to study the functional impact of gut microbiome. Overall, the donor accounted for 43% of the differences in the circulating metabolome, while the severity of the cholestatic disease in the mice explained up to 7% of the differences. However, the composition of the metabolome was not associated with the donor's disease status.

Conclusion: Despite limited differences between PSC and HC donors, the microbiota of humanized mice seems to recapitulate the donor's microbiota and has a major impact on metabolic profile. Overall, this suggests that our study design is feasible but that

PSC donors with a higher degree of gut dysbiosis may be needed to exacerbate liver injury in mice.

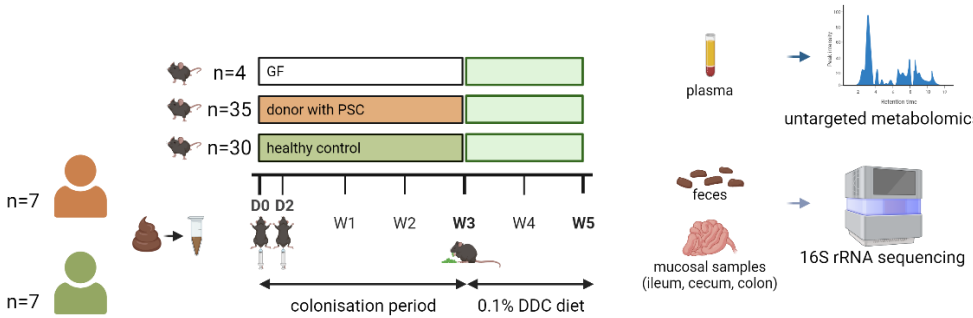

Fig. 1: Detailed experimental design (D, day; W, week; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine) (created by BioRender).

Global metabolomics in the mouse and pig brain

Anselmo Cde S[1*], Vallianatou T[2], Tsiara I[1], Lundgaard I[3,4], Globisch D[1]

*[1]Department of Chemistry BMC, Uppsala University, Uppsala, Sweden. * E-mail: carina.anselmo@kemi.uu.se; [2]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; [3]Department of Experimental Medical Science, Lund University, Lund, Sweden; [4]Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden.*

The mental health of the population has become an increasing matter of social and economic relevance, especially after the COVID-19 pandemic[1]. The gut-brain axis has emerged as an important factor to consider in the development of mental disorders due to up- and down-regulations in the composition of the gut microbiota in mental diseases.[2] Due to the blood-brain barrier, the flux of molecules towards and from the brain tissue are strictly controlled to limit exposure of the brain to potentially neurotoxic molecules.[3] Thus, the knowledge about brain metabolism in essential for looking for disease biomarkers. In previous work, our group demonstrated a strong correlation between circadian rhythms and specific amino acid metabolism for different mice brain regions.[4] Nowadays, the investigation has been made in pigs due to their similar diet and metabolism to humans in contrast to rodent models.[5] Before euthanasia, the pigs were under continuous anesthesia with a mixture of ketamine, fentanyl, and midazolam. Therefore, the goal of this work is to evaluate by global metabolomics and targeted analysis by LC-HRMS the flux of endogenous and exogenous metabolites between different regions of the pig brain. The different distribution between the pig's brain regions regarding some phase II metabolites from ketamine and midazolam was observed, but not for phase I metabolites. We observed different distributions in the pig brain regions of several endogenous and exogenous phase II metabolites. By expanding the understanding of substance metabolism in the pig brain we intend to contribute to the search for biomarkers for mental illness.

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Gut microbiota diversity is associated with progression-free survival in metastatic triple-negative breast cancer

Ullern A[1,2], Holm K[3,4,5], Røssevold AH[1,2], Andresen NK[1,2], Bang C[6], Hov JR[3,4,5,7], Kyte JA[1,2]

[1]Department of Clinical Cancer Research, Oslo University Hospital, Oslo, Norway; [2]Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [3]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [4]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; [5]Norwegian PSC Research Center, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway; [6]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany; [7]Section of Gastroenterology, Department of Transplantation Medicine, Oslo University Hospital, Oslo, Norway.

Background: The gut microbiota is associated with response and toxicity to immune checkpoint inhibitors in multiple cancer types, but there is paucity of data on metastatic triple-negative breast cancer (mTNBC).

Methods: In the randomized phase IIb trial ALICE patients with mTNBC were randomized 2:3 to pegylated liposomal doxorubicin and cyclophosphamide alone (placebo-chemo) or combined with atezolizumab (atezo-chemo). Fecal samples were collected at baseline (atezo-chemo *n*=36, placebo-chemo *n*=23) and after 8 weeks (atezo-chemo *n*=31, placebo-chemo *n*=18). 16S (v3-4) rRNA sequencing was applied to characterize the diversity and taxonomic composition of the gut microbiota. ANCOM-BC2 was used for differential abundance analysis at the genus level. Kaplan-Meier methods and Cox proportional hazard models were used to test the relationship between alpha diversity and progression-free survival (PFS).

Results: High alpha diversity at baseline was associated with prolonged PFS in all patients (HR 0.56, 95% CI 0.33-0.98, *P* = 0.04). No association between alpha diversity and immune-related adverse events was observed. In all patients, alpha diversity was significantly reduced after 8 weeks of treatment. At baseline, *Bifidobacterium* was significantly overrepresented in patients without clinical benefit in the atezo-chemo arm, but not in the placebo-chemo arm. *Bifidobacterium* significantly increased after 8 weeks of treatment in the atezo-chemo arm, but only in patients with clinical benefit.

Conclusion: High alpha diversity was associated with prolonged PFS in metastatic TNBC patients. These data suggest alpha diversity may serve as a prognostic biomarker in this setting.

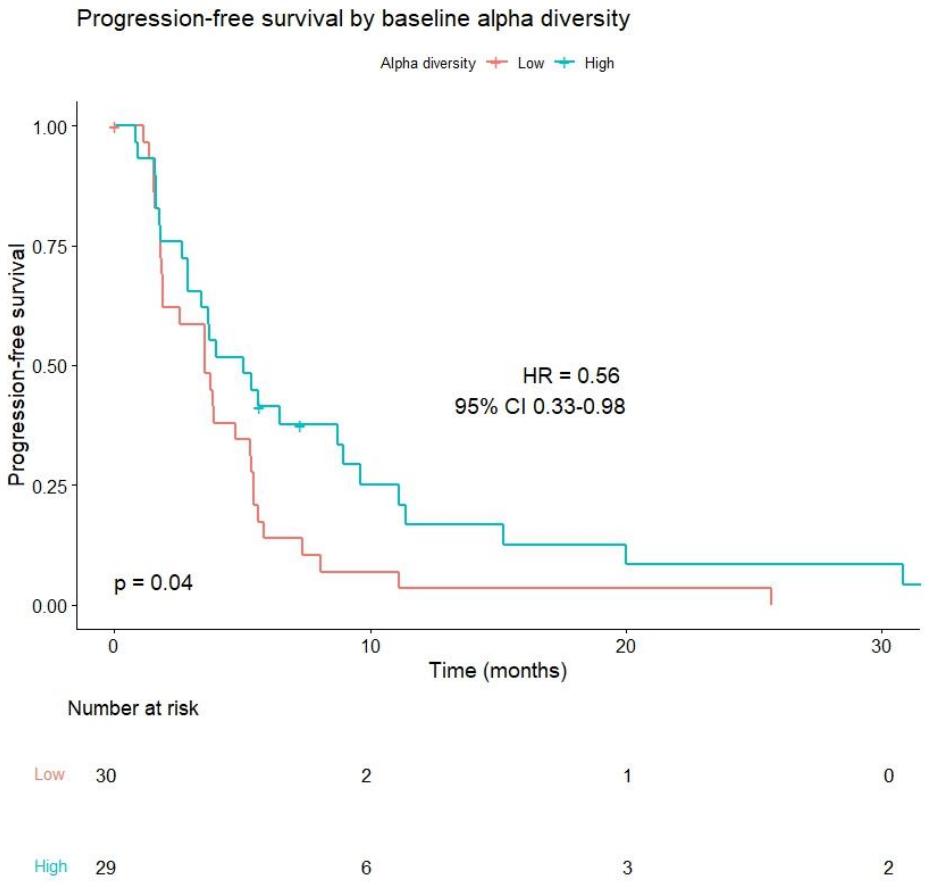

Fig. 1: Kaplan-Meier plot of PFS by baseline gut diversity (Faith's PD) in all patients. Patient were classified into low and high diversity groups based on the median score of Faith's PD.

Alcohol intake, gut microbiome and colorectal carcinogenesis

Kværner AS[1], Birkeland E[2], Bucher-Johansen C[3,4], Botteri E[1], Rounge TB[3,4,5], Berstad P[1]

[1]Section for Colorectal Cancer Screening, Cancer Registry of Norway, Oslo, Norway; [2]Centre for Bioinformatics, Department of Informatics, University of Oslo, Oslo, Norway; [3]Department of Tumor Biology, Institute of Cancer Research, Oslo University Hospital, Oslo, Norway; [4]Department of Research, Cancer Registry of Norway, Oslo, Norway; [5]Centre for bioinformatics, Department of Pharmacy, University of Oslo, Oslo, Norway.

Background: Alcohol consumption is one of the major risk factors of colorectal cancer (CRC). However, the mechanisms underlying this relationship are not fully understood, particularly the role of gut microbes.
Objective: To study associations between alcohol intake, gut microbiome and colorectal carcinogenesis using data from a large bowel cancer screening trial in Norway (BCSN-CRCbiome).
Design: Fecal immunochemical test positive participants, aged 55-77 years, were included in this cross-sectional investigation. Intake of alcohol was assessed using a validated, semi-quantitative food frequency questionnaire and combined with metagenomic based taxonomic and functional profiles to study associations with screen-detected colorectal lesions.
Results: Of 1,468 participants, 414 were diagnosed with advanced lesions (advanced precursor lesions or CRC). Alcohol intake was positively associated with advanced lesions in a dose response manner (p_{trend} of 0.008), with OR (95% CI) per 2-fold increase of 1.14 (1.05, 1.23). Compared to the non-consumers, those consuming alcohol were characterized by a distinct microbial profile, manifested as higher α-diversity (3 and 10% by means of the Shannon and Inverse Simpson indices), altered microbial composition (PERMANOVA p-value 0.004) and differentially abundant bacteria (n=6) and pathways (n=7). As for alcohol intake, alcohol-associated bacteria were positively associated with advanced lesions (p_{trend} of <0.001). Adjusting or stratifying the main analysis for these bacteria did not alter the association between alcohol intake and advanced lesions.
Conclusion: While consumers of alcohol have a distinct microbial profile associated with advanced colorectal lesions, the microbial pathway likely represents only one of several mechanisms whereby alcohol may promote colorectal carcinogenesis.

Microbiota profiling as a diagnostic and prognostic tool: Insights from the IBSEN III cohort

Hansen SH[1,2,3,*], Maseng MG[2,4,5,*], Grännö O[6], Halfvarson J[7], Ricanek P[8], Detlie TE[9,10], Huppertz-Hauss G[11], Kristensen VA[4], Opheim R[4,12], Tønnessen T[13], Perminow G[14], Bengtson MB[15], Cetinkaya RB[16], Lund C[2,4], Hovde Ø[2,17], Medhus AW[2,4], Frigstad SO[18], Strande V[2,8,19], Valeur J[2,19], Vatn S[10], Aabrekk TB[15], Høivik ML[4#], Hov JR[1,2,3,20#]

[1]Norwegian PSC Research Center, Dep. of Transplantation Medicine, Division of Surgery, Inflammatory Diseases and Transplantation, Oslo University Hospital, Oslo, Norway; [2]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [3]Research Institute of Internal Medicine, Division of Surgery, Inflammatory Diseases and Transplantation, Oslo University Hospital, Oslo, Norway; [4]Dep. of Gastroenterology, Oslo University Hospital, Oslo, Norway; [5]Bio-Me, Oslo, Norway; [6]School of Medical Sciences, Örebro University, Örebro, Sweden; [7]Dep. of Gastroenterology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden; [8]Dep. of Medicine/Gastroenterology, Lovisenberg Diaconal Hospital, Oslo, Norway; [9]University of Oslo, Oslo, Norway; [10]Dep. of Gastroenterology, Akershus University Hospital, Lørenskog, Norway; [11]Telemark Hospital, Dep. of Gastroenterology, Skien, Norway; [12]Dep. of Public Health, Institute of Health and Society, University of Oslo, Norway; [13]Dep. of Gastroenterology, Drammen Hospital, Vestre Viken Hospital; [14]Pediatric Dep., Oslo University Hospital, Oslo, Norway; [15]Medical Dep., Vestfold Hospital Trust, Tønsberg, Norway; [16]Dep. of Medicine, Diakonhjemmet Hospital, Oslo, Norway; [17]Dep. of Medicine, Innlandet Hospital Trust, Gjøvik, Norway; [18]Dep. of Medicine, Bærum Hospital, Vestre Viken Hospital Trust, Norway, [19]Unger-Vetlesen Institute, Lovisenberg Diaconal Hospital, Oslo, Norway; [20]Section of Gastroenterology, Dep. of Transplantation Medicine, Division of Surgery, Inflammatory Diseases and Transplantation, Oslo University Hospital, Oslo, Norway.
**Shared first authorship*
 # Shared senior authorship

We aimed to determine the diagnostic and prognostic potential of microbiota profiling in persons with suspected inflammatory bowel disease (IBD). To achieve this, we 16S sequenced 1404 stool samples from participants of the prospective population cohort IBSEN III.

After standardized diagnostic work-up, patients were classified as: Ulcerative colitis (UC, 50%), Crohn's disease (CD, 29%), IBD-U (3%) suspected IBD (6%) or non-IBD symptomatic control (12%).

IBD subtype: UC and CD patients had distinct microbial compositions (*PERMANOVA $p<0.0001$*), and 27 taxa were differentially abundant between them (validated with three different methods; *MaAsLin2, ANCOM-BC2 and LinDA; FDR-adjusted $p< 0.05$*). An UC-CD microbial index based on these taxa discriminated between ileal and colonic CD (*$p=0.006$*), while simultaneously separating colonic CD from UC (*$p<0.0001$*). This indicates that microbiota profiling can be used as a non-invasive tool for assessing the location and type of IBD prior to colonoscopy.

Risk of severe disease: Microbiota profiles outperformed biomarkers (e.g. CRP, calprotectin and others) in predicting the future onset of a severe disease course in UC

patients (*median AUC 0.72 vs 0.65, p<0.0001*). Combining both data types did not improve prediction performance, even when patients with severe inflammation at inclusion were excluded, suggesting that the microbial predictive capacity is partly independent of inflammatory burden at diagnosis.

Conclusion: Based on a prospective population cohort of newly diagnosed IBD patients and symptomatic controls, microbiota profiling is promising as a non-invasive diagnostic and prognostic tool for early IBD classification and high-risk detection, outperforming commonly used biomarkers such as calprotectin and CRP.