

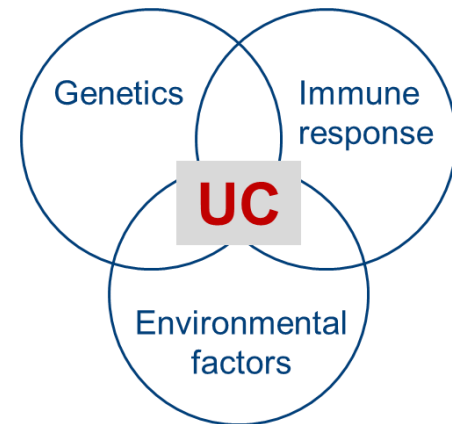
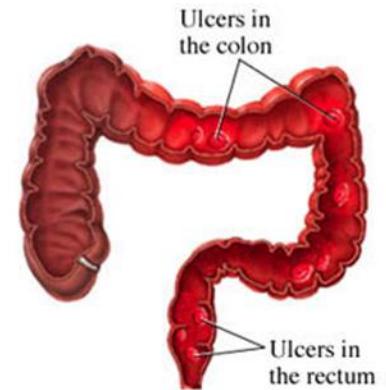


Altered composition of colonic luminal compounds in patients with ulcerative colitis

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27 November 2014
16th Gut Day Symposium

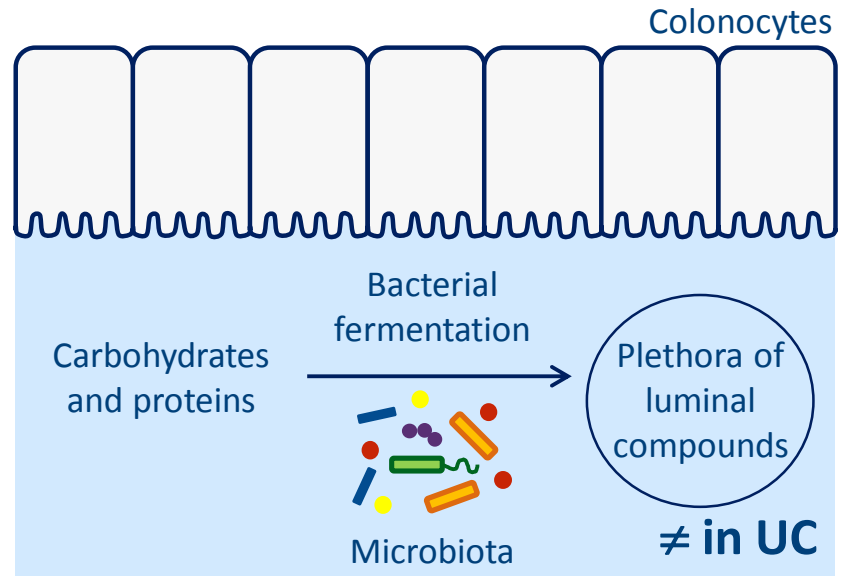
Ulcerative colitis (UC)

- Chronic inflammation of the colonic mucosa
 - Alternating periods of active and quiescent disease
- Symptoms: - ulcers, bloody diarrhea and abdominal pain
- increased risk of colorectal cancer (CRC) ¹
- Prevalence in Europe: 4.9 - 505 per 100,000 persons ²
- Underlying etiology = multifactorial ³
- Environmental factors
 - Smoking: protective factor
 - Diet: animal fat, high protein, alcoholic beverages
 - Microbiota



Microbiota in health and UC

- Human colon ¹
 - 10¹⁴ bacteria
 - Metabolic function
 - Carbohydrate fermentation
 - Protein fermentation
- Dysbiosis in UC ²
 - Biodiversity ↓
 - Firmicutes ↓
 - *Lactobacillus*, *Bifidobacterium* ↓
 - *Clostridium* clusters IV & XIVa ↓



- Study aims: 1) Determine the metabolic activity in health and UC
2) Associate the metabolome with cytotoxicity (as a CRC marker)

Study design

- Faecal samples were collected from:
 - Healthy controls (HC)
 - UC patients with active disease (UC-A)
 - UC patients in remission (UC-R)
- Disease activity was determined by the partial Mayo score (range 0-9):
 - Active (UC-A): pMayo ≥ 3
 - Remission (UC-R): pMayo ≤ 2
- Preparation of faecal water (FW):
 - Faecal samples were centrifuged at 50,000 g at 4 °C for 2 h
 - Supernatant was sterile filtered through a 0.22- μ m filter
- The volatile organic compounds (VOCs) were determined using GC-MS and cytotoxicity of the samples using a colorimetric method

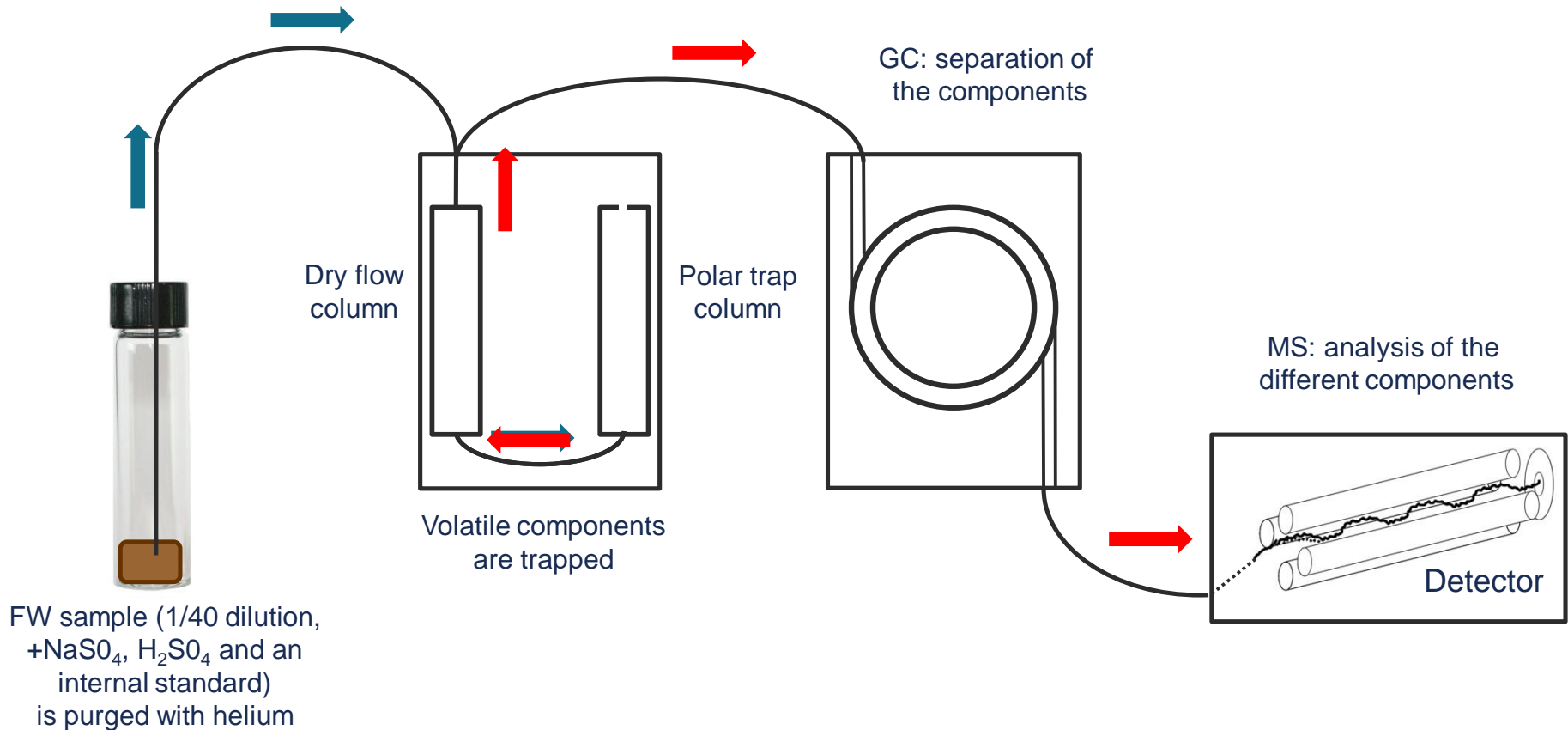
Cohort characteristics

	HC N=52	UC-A N=58	UC-R N=60	P-value
Age (years)	38 (\pm 16)	46(\pm 16)	47(\pm 16)	0,001
BMI (kg/m ²)	24 (\pm 3)	25 (\pm 3)	25 (\pm 3)	0,376
Male/female (%)	37/63	60/40	57/43	0,028

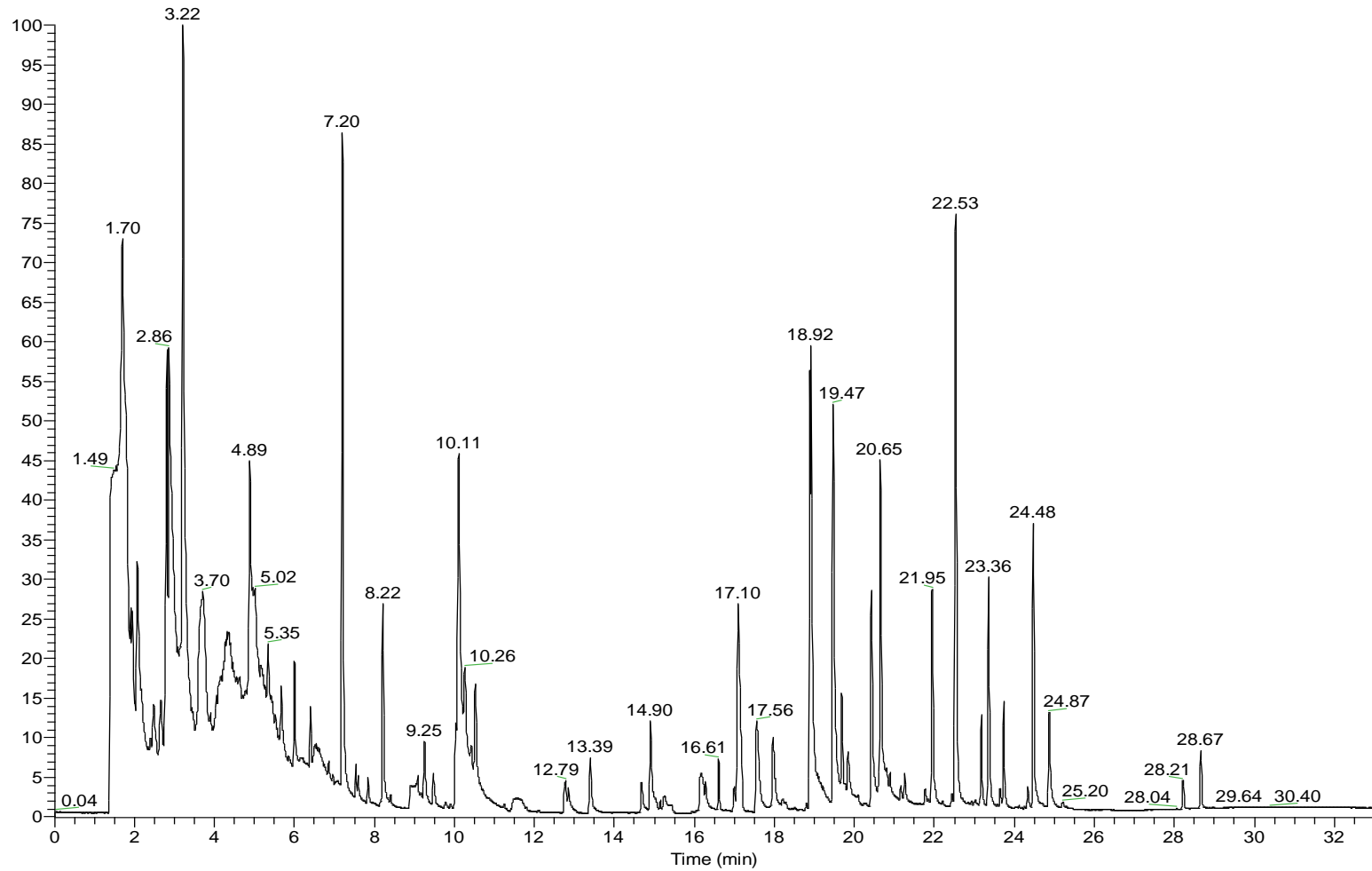
- Exclusion criteria:
 - Surgery of the gastrointestinal tract
 - Colonoscopy in the preceding month
 - Antibiotic use in the preceding month
 - UC patients: disease extent limited to the rectum

GC-MS analysis results in metabolite profiles

- All VOCs were relatively quantified versus an internal standard and classified according to chemical class

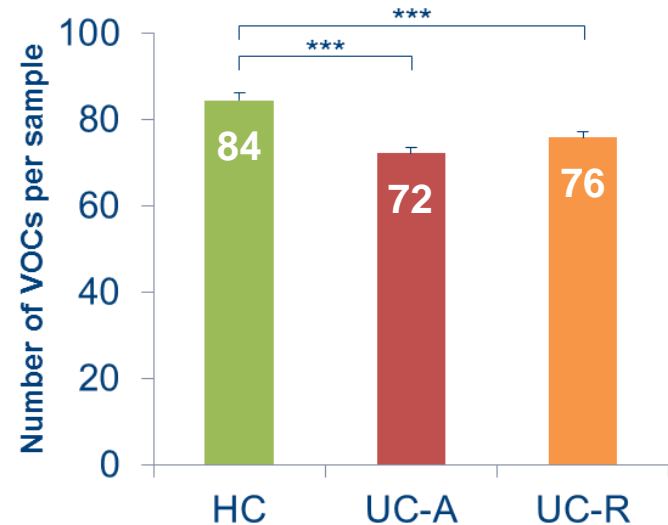


Typical metabolite pattern of a faecal sample

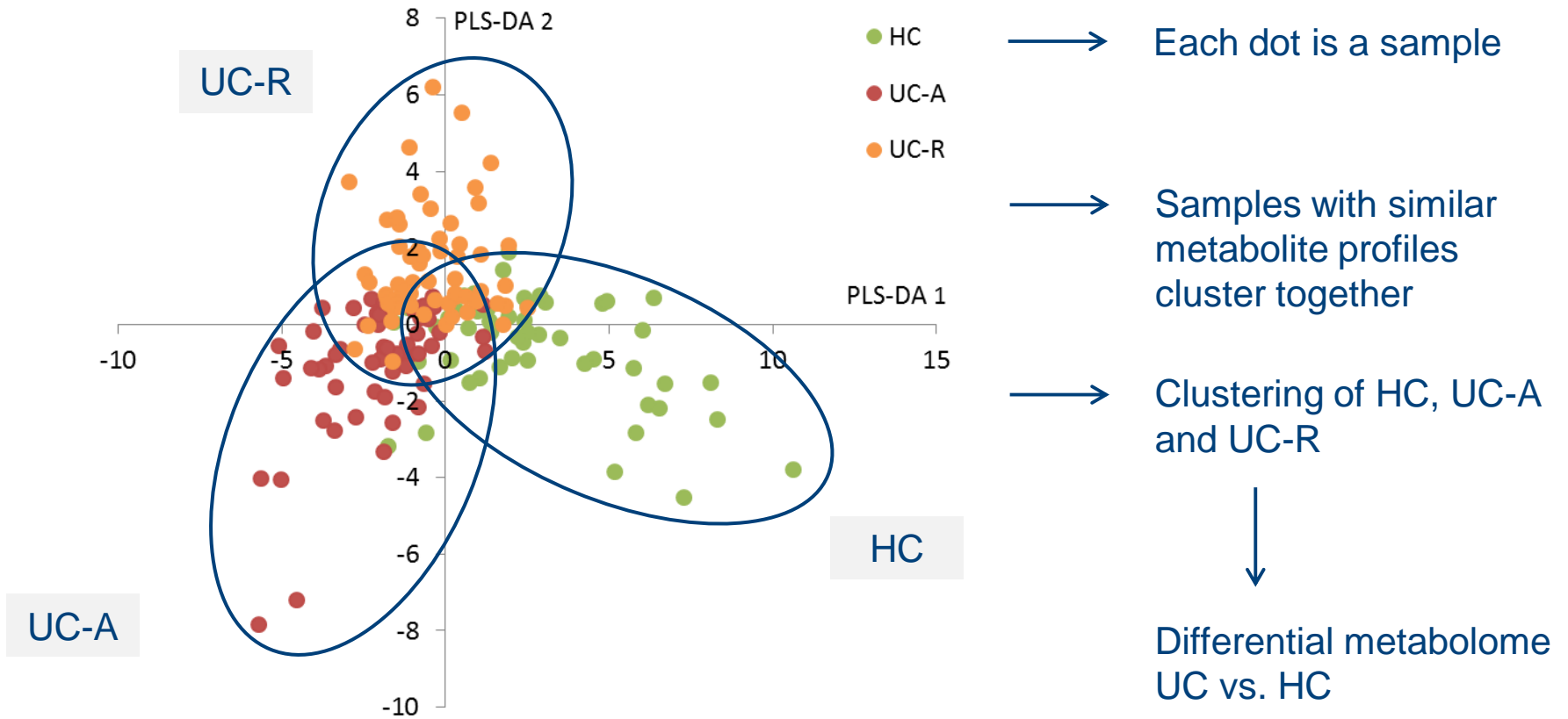


Results metabolome analysis

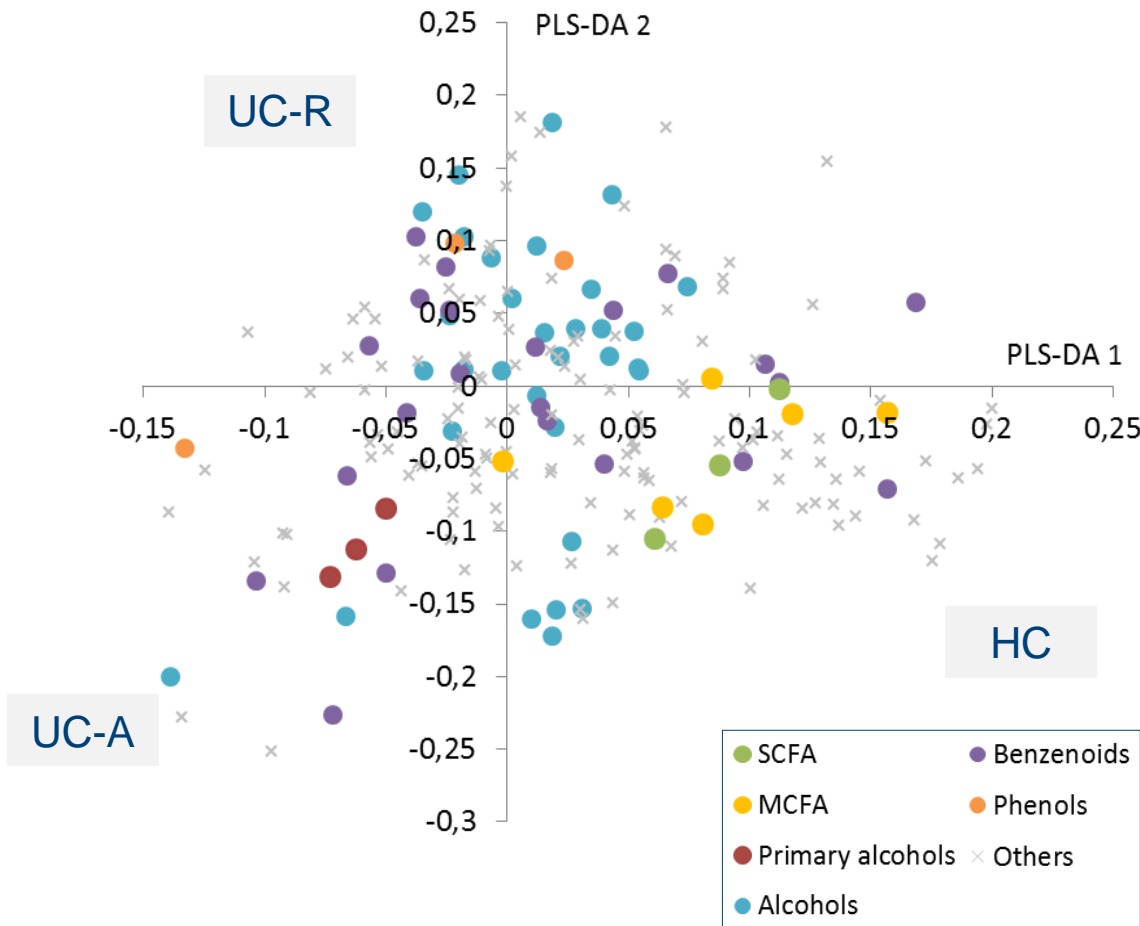
- 237 VOCs were identified in the FW samples:
 - Average: 77 (± 1) / sample
 - Minimum: 45 / sample
 - Maximum: 108 / sample
 - 27 sample-specific VOCs
 - 11 VOCs in all samples
- UC patients have significantly less VOCs per sample than HC



PLS-DA score plot showing all FW samples



PLS-DA loading plot showing all VOCs



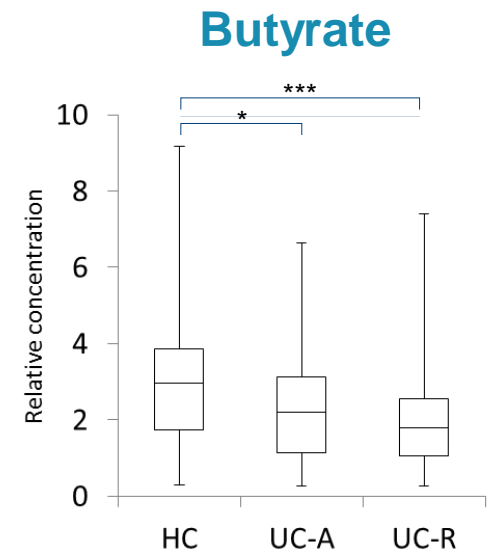
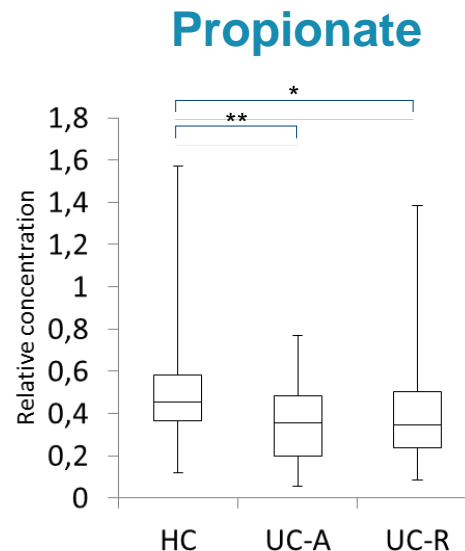
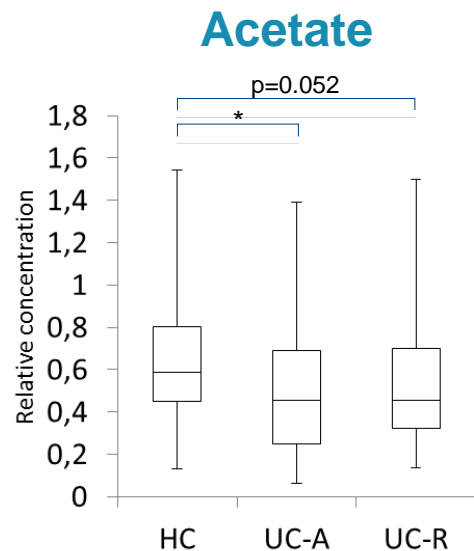
→ Each dot is a metabolite

→ Metabolites away from the center are important for the separation

→ Metabolites in the lower right quadrant are associated with HC samples

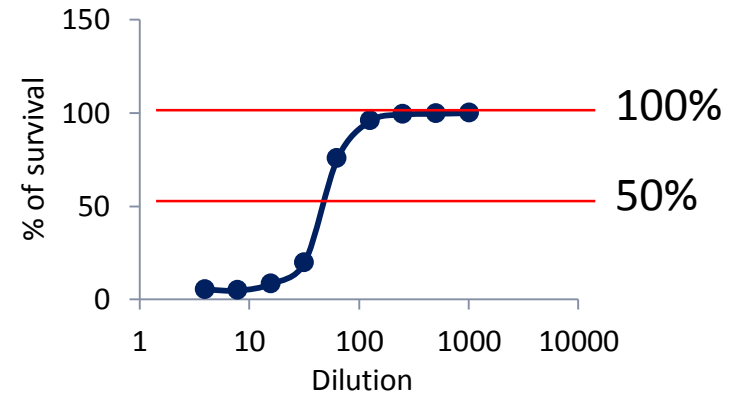
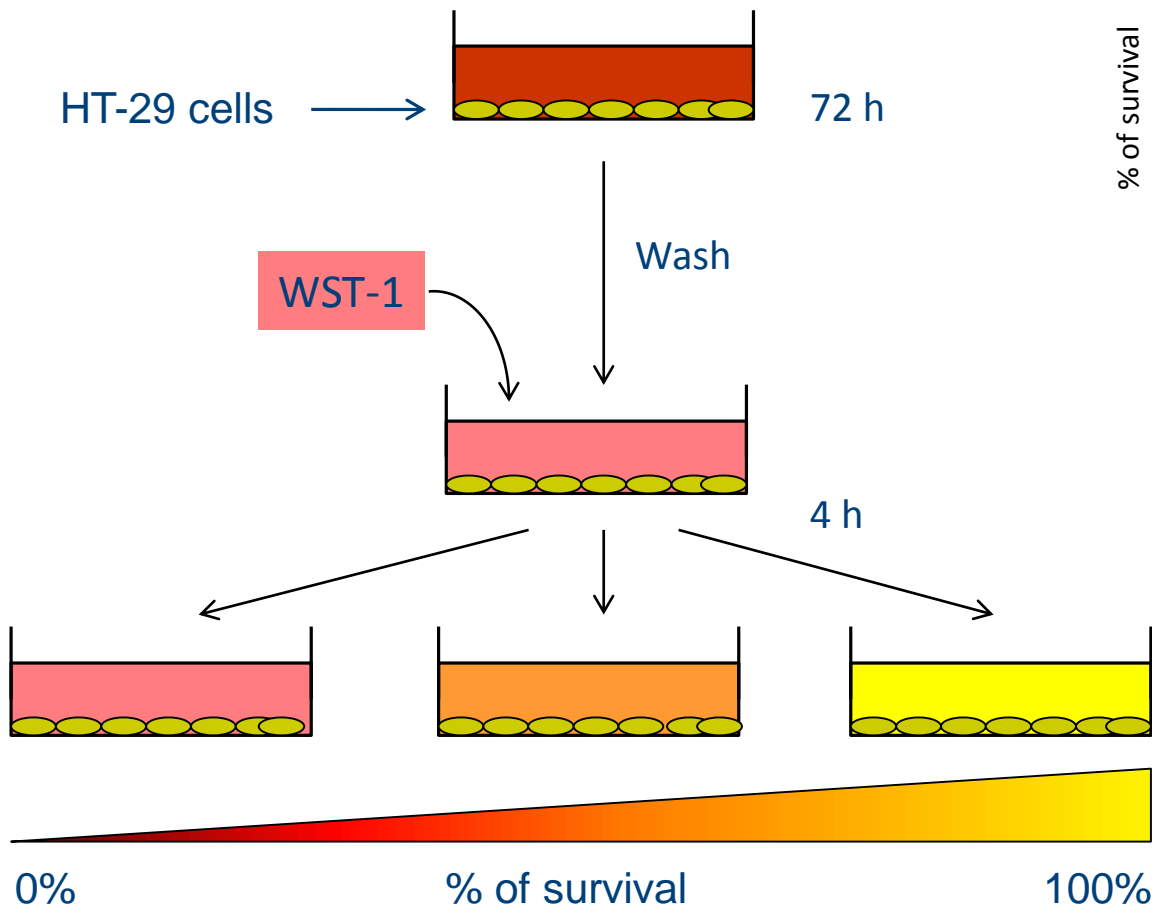
VOCs associated with sample groups

- FW samples from UC patients were associated with:
 - Higher prevalence of:
 - Alcohols (Primary alcohols for UC-A)
 - Phenols
 - Benzene-derivatives } Protein fermentation metabolites
 - Lower prevalence of:
 - Short chain fatty acids (SCFA) } Carbohydrate fermentation metabolites
 - Medium chain fatty acids (MCFA)



Impact of the metabolome on cytotoxicity

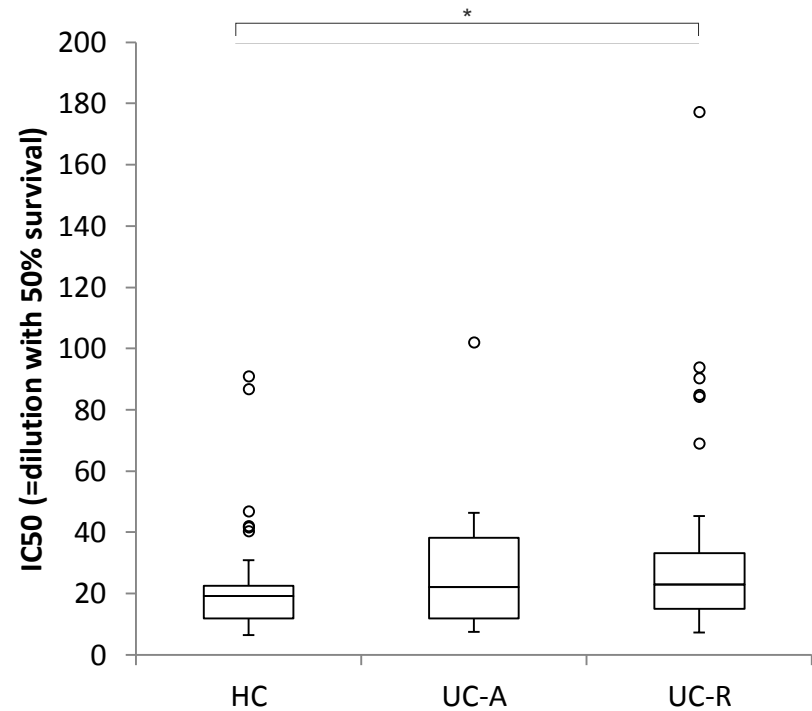
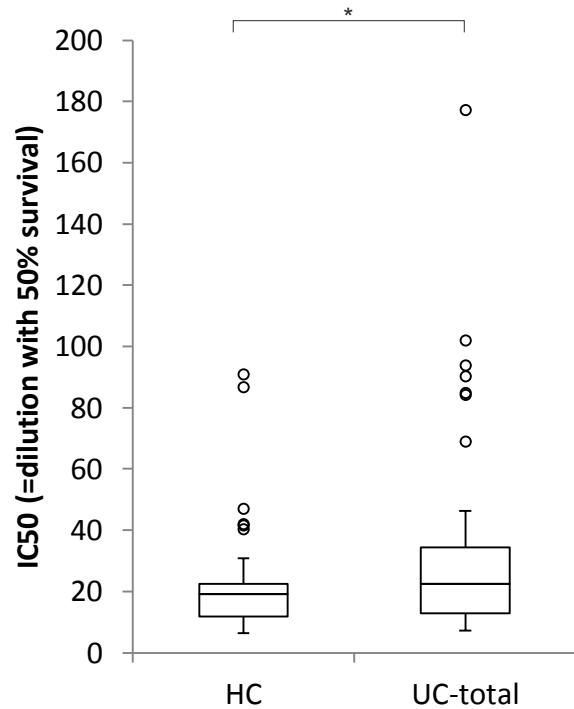
Incubation with faecal water



IC_{50} =
Dilution at which 50%
of cells survive

High IC_{50} =
More cytotoxic

FW samples from UC patients are more cytotoxic



* $p < 0.05$

VOCs associated with cytotoxicity

- FW samples with higher cytotoxicity were associated with:
 - Higher prevalence of:
 - Furans
 - Aldehydes
 - Branched-chain fatty acids (BCFA)
 - S-containing compounds

} Protein fermentation metabolites
 - Lower prevalence of:
 - SCFA
 - MCFA

} Carbohydrate fermentation metabolites

Conclusions

- The faecal metabolite profiles allowed to differentiate HC from UC patients
- Most discriminatory metabolites:
 - For UC-A: primary alcohols
 - For HC: SCFA and MCFA
- FW samples from UC (especially UC-R) patients are more cytotoxic than HC samples
 - This could contribute to the higher CRC risk for UC patients and, through barrier disruption, to the induction and/or continuation of inflammation
- Promoting carbohydrate over protein fermentation could positively affect the colonic health in UC
 - Can be achieved by the use of prebiotics or diet adjustments

Acknowledgements

Prof. Kristin Verbeke
Prof. Séverine Vermeire
Dr. Karen Windey
Dr. Vicky De Preter
Greet Vandermeulen
Anja Luypaerts
Els Houben
Eef Boets
Lise Deroover

