Exploring the Coeliac Fermentome and Microbiome

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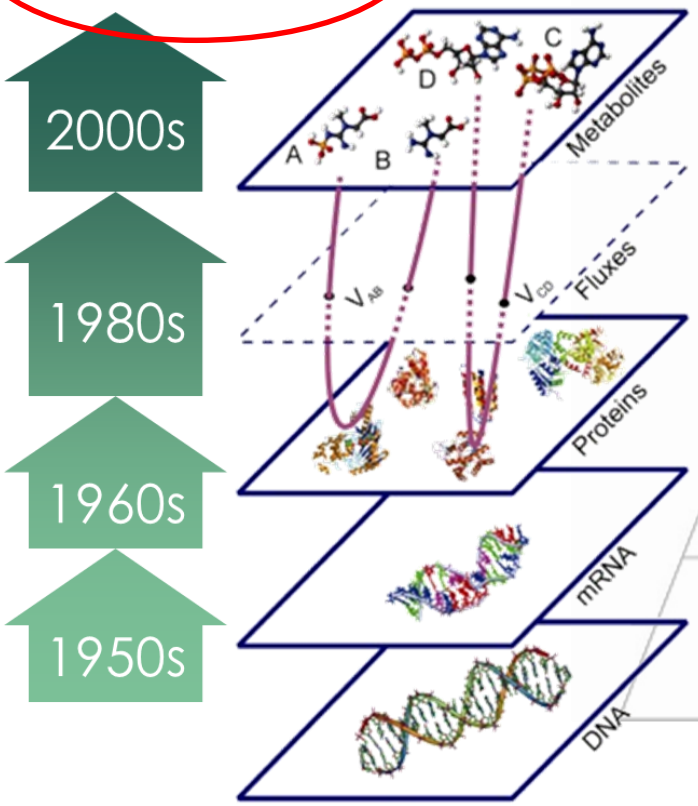
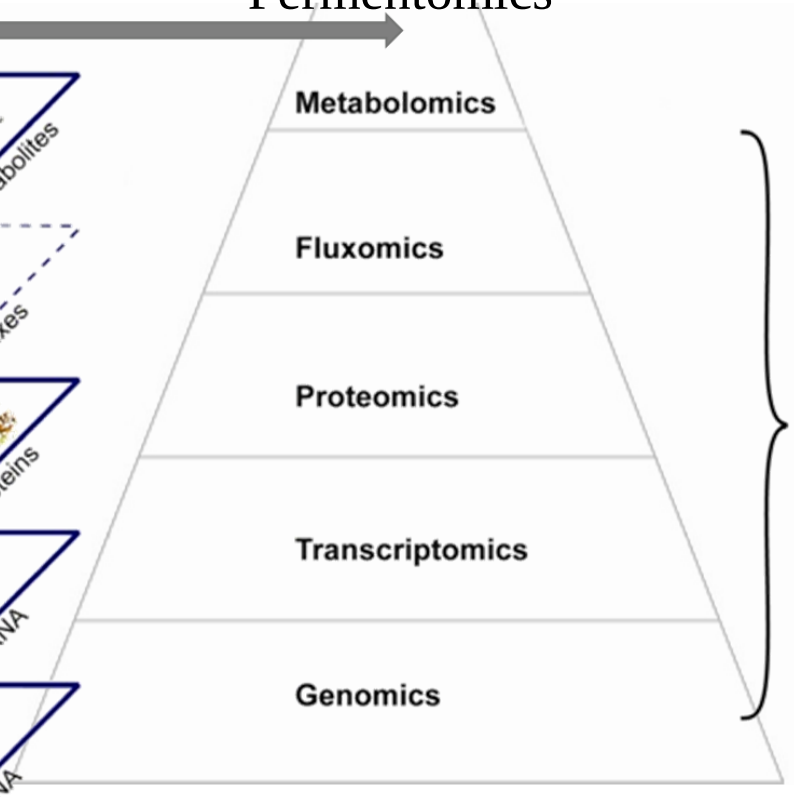
Omics *'frenzy'*

Volatile markers

Fermentomics





System biology

Integrative physiology

System medicine

System pharmacology

Regenerative medicine

Integrated biomarkers

Human disease
- Prediction
- Diagnostics
- Treatment efficacy

Figure adapted from Nemutlu E et al. 2011;53:529–534

Volatile organic compounds (VOCs)

- Disease alters gut flora

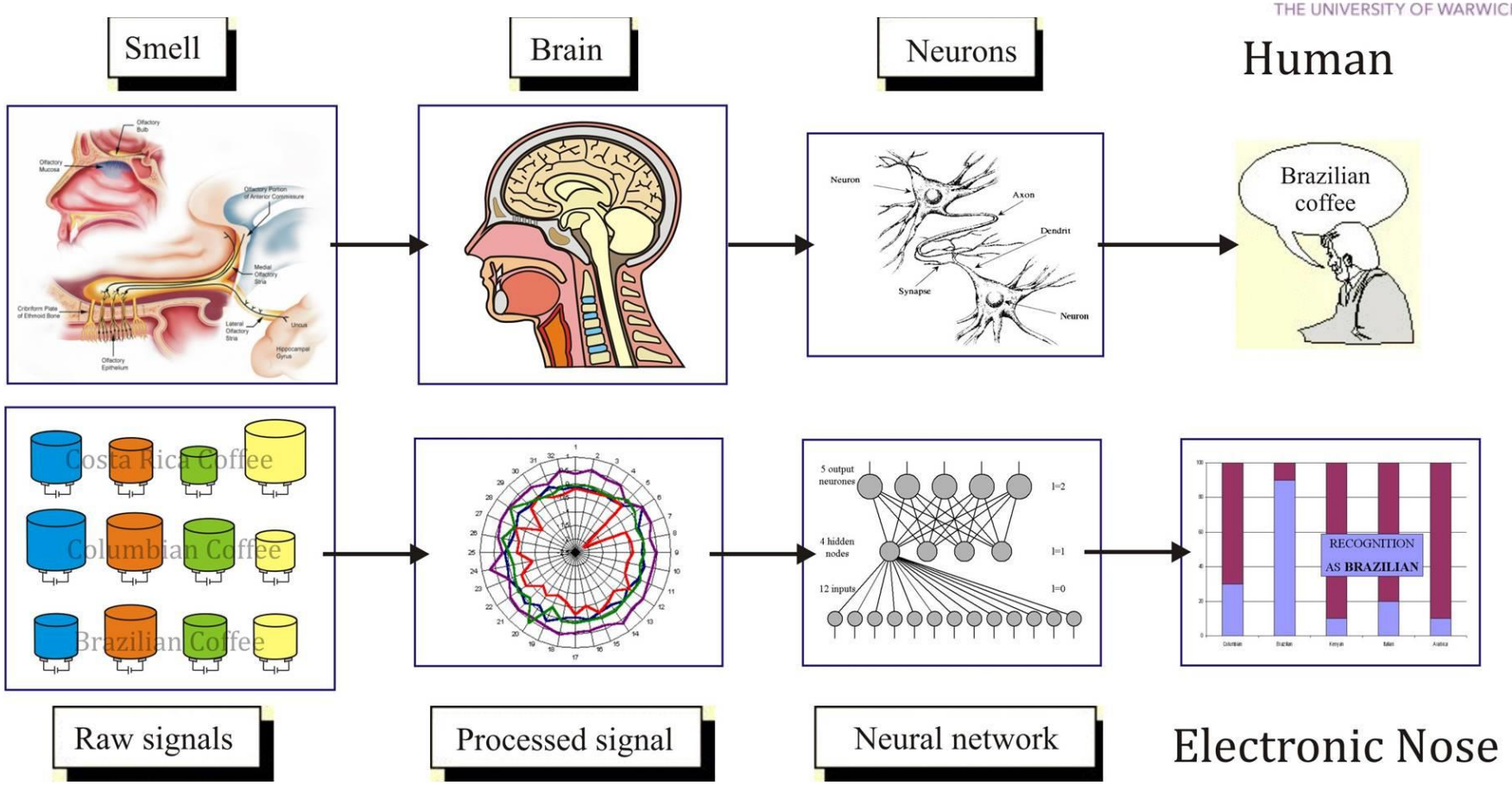
– ‘dysbiosis’ with altered fermentation patterns

- Organic compounds – in the gas phase
- Colonic fermentation by gut bacteria/ physiological metabolic processes

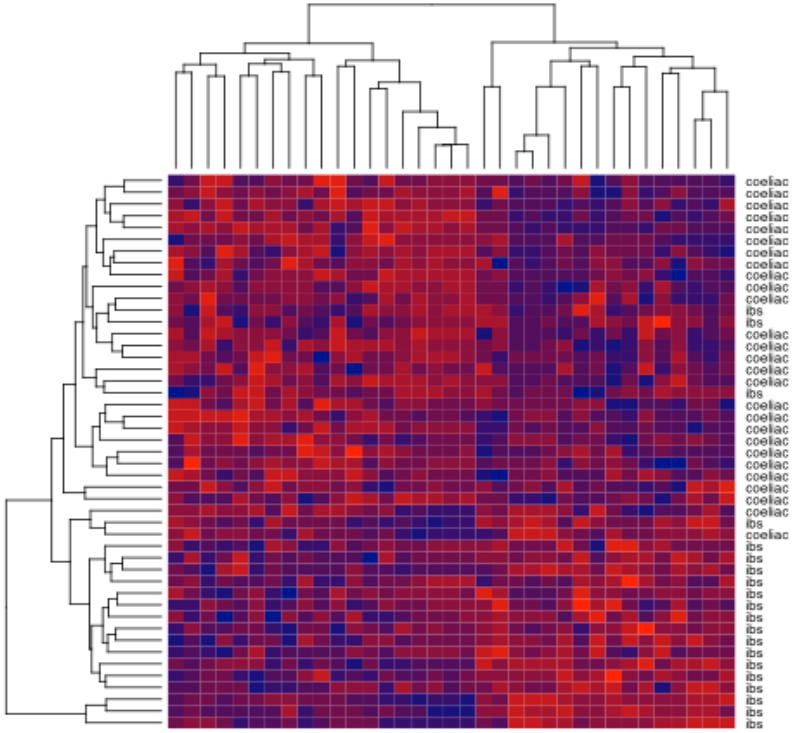
- Released in breath, urine, faeces, blood, sweat

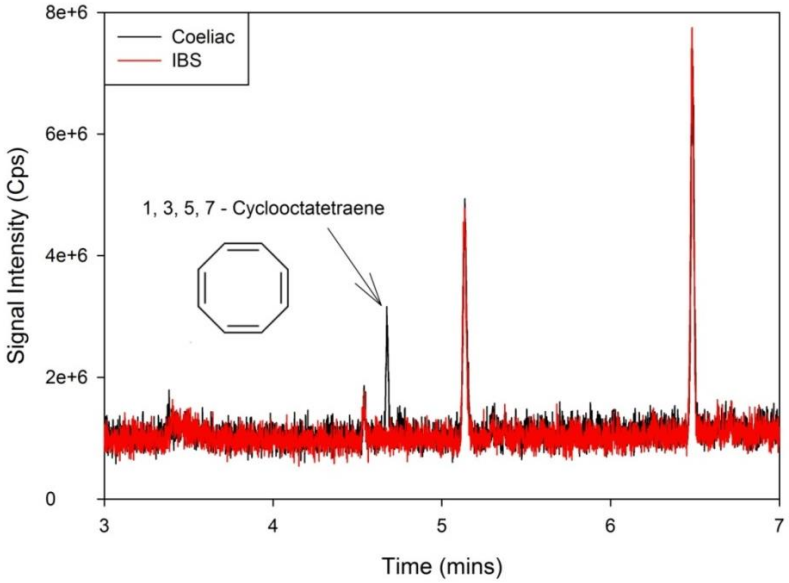
Arasaradnam RP et al. Dig Liver Dis 2016;48:148–153.

Instrumentation for detecting Volatile Compounds (electronic nose)



Urine VOC for diagnosis of coeliac disease





Arasaradnam et. al. 2014

EU/USA Patent Office: 2015

Fermentome and Microbiome study

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Exploratory study

- Changes to the VOC signal in response to gluten
    - What were the chemicals
- Changes in the microbiome
    - Was there a shift in pattern
- Immune regulated genes & microbiome

CD (n=16)
(pre-treatment)

6 months

coeliac 1

coeliac 2

-Symptoms
-Raised tTG
-Confirmatory duodenal histology

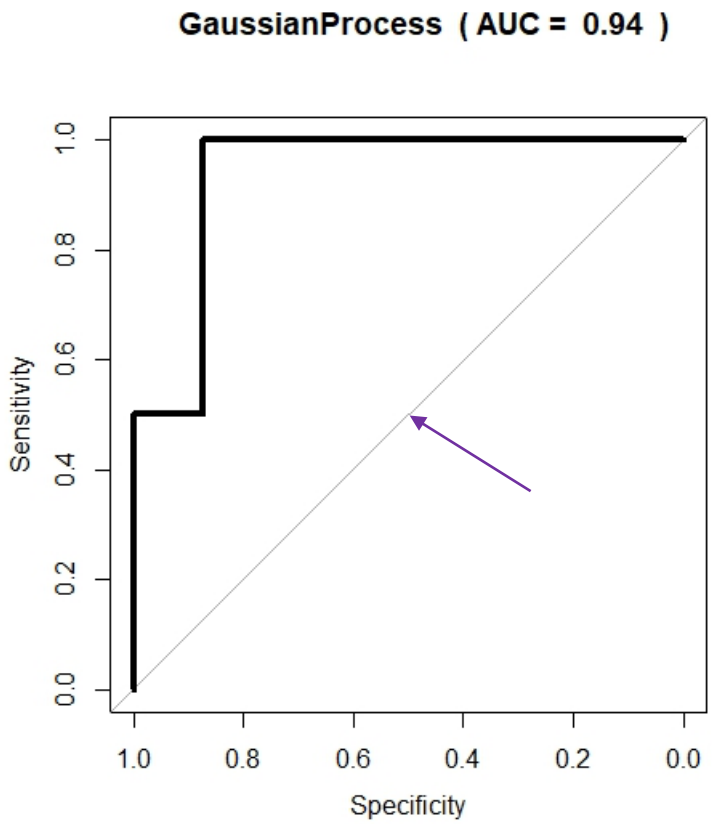
-Symptoms/anaemia resolved
-Histological resolution

Controls (n=18)

control 1: raised tTG but normal histology; DQ2/DQ8 positive in 50%
control 2: normal tTG and histology
control 3: normal tTG

URINE VOC (VOLATILE ORGANIC COMPOUNDS)

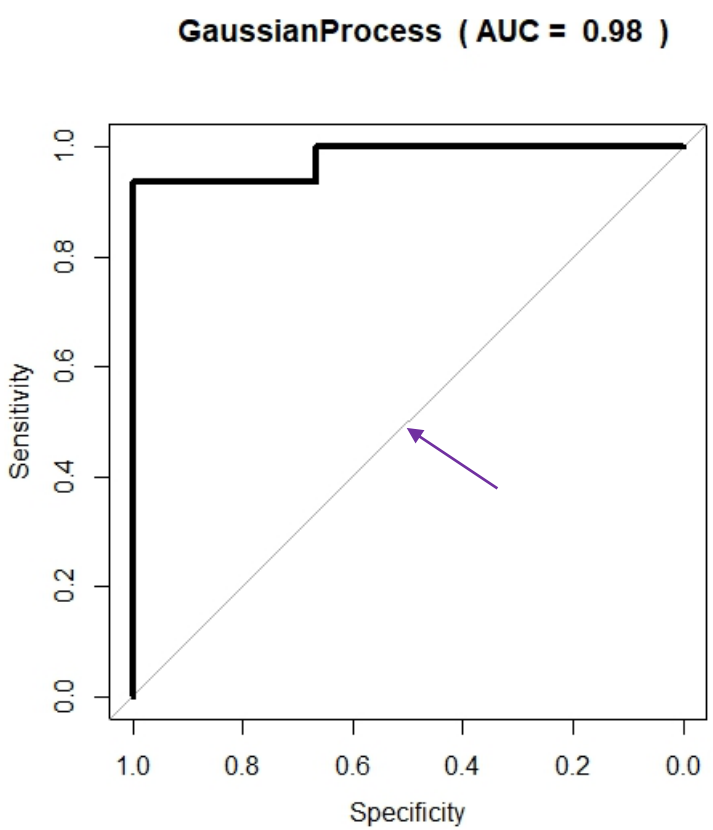
Urine VOC – separation of coeliac disease patients pre diagnosis and 6 months later



- AUC = 0.94 (0.81 - 1)
- sensitivity = 1 **(0.63 - 1)**
- specificity = 0.88 **(0.47 - 1)**
- PPV = 0.89
- NPV = 1

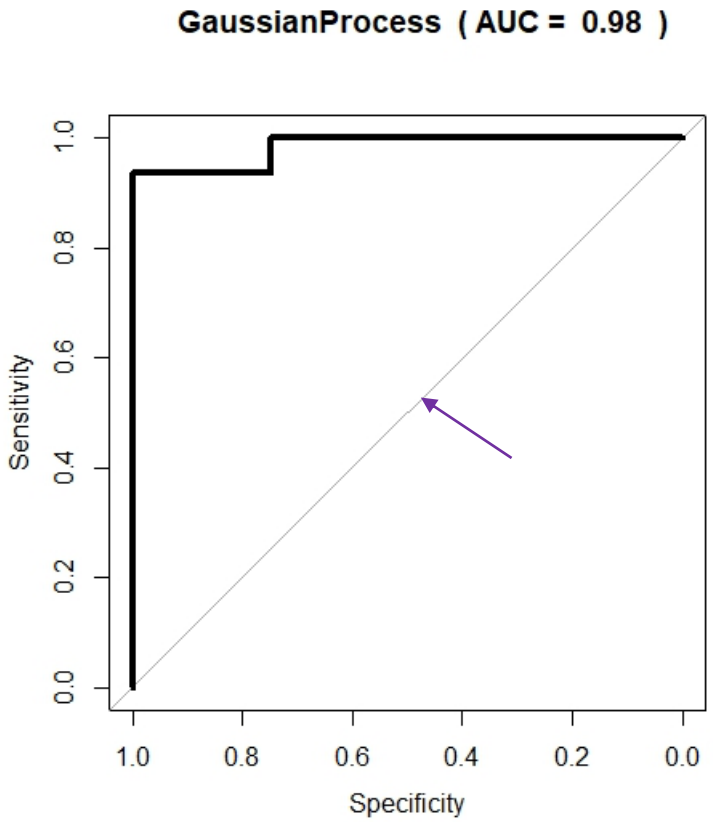
Urine VOC – separation of coeliac disease patients from control 1 and 2

Control 1



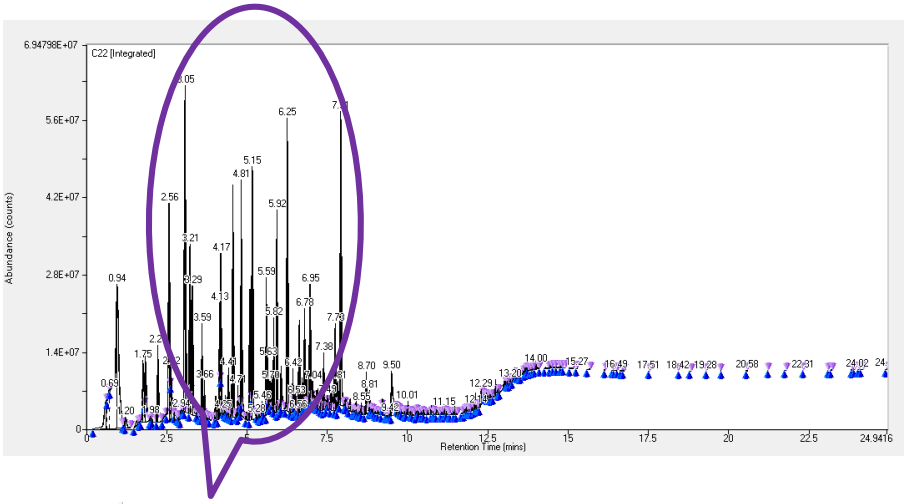
sensitivity = 0.94 (0.7 - 1)
specificity = 1 (**0.54 - 1**)
PPV = 1
NPV = 0.86

Control 2

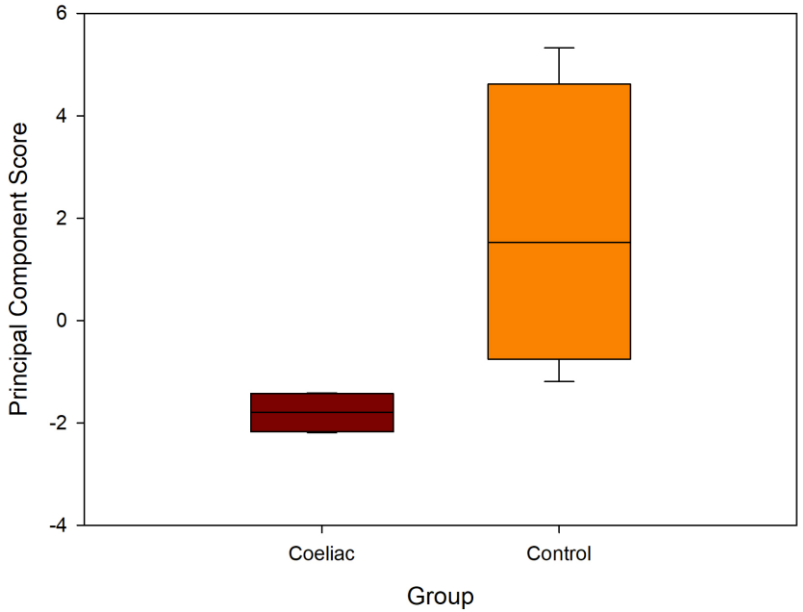


sensitivity = 0.94 (0.7 - 1)
specificity = 1 (**0.63 - 1**)
PPV = 1
NPV = 0.89

Chemical Identification – GC IMS



Compound Name	Mass	RT	Start	Stop	Height	Width	Group	Area	Area %
Carbon dioxide	TIC	1.6014	1.1694	1.625	1.1414E+06	1.4556		1.52868E+07	1.50
Carbon dioxide	TIC	1.6948	1.625	1.7139	7.09386E+06	1.0889		1.04395E+08	1.64
Oxalic acid	TIC	1.9943	1.7139	1.125	1.5987E+07	1.4111		1.60881E+08	11.03
2-Pentanamine, 4-methyl-	TIC	1.139	1.125	1.1639	1.96792E+05	1.0389		1.597E+06	0.06
Acetaldehyde	TIC	1.2033	1.1639	1.3694	1.01722E+06	1.2056		1.17929E+07	0.34
Silane, methyl-	TIC	1.5703	1.4583	1.6139	1.40414E+05	1.1556		1.00803E+07	0.16
Cyclopropyl carbinol	TIC	1.6393	1.6139	1.6583	1.10365E+05	1.0444		1.92692E+05	0.01
Acetone	TIC	1.752	1.6583	1.7917	1.12506E+07	1.1333		1.90086E+07	1.40
Isopropyl Alcohol	TIC	1.8233	1.7917	1.9472	1.20053E+07	1.1556		1.1878E+08	1.87
Methylene chloride	TIC	1.9762	1.9472	2.0028	1.00571E+05	1.0555		1.28993E+06	0.08
3,4-Epoxytetrahydrothiophene-1,1-dioxide	TIC	2.015	2.0028	2.1528	6.06924E+05	1.15		5.89918E+06	0.06
Propanal, 2-methyl-	TIC	2.212	2.1528	2.3417	1.39436E+07	1.1889		1.01717E+08	1.60
Glycine	TIC	2.461	2.3417	2.475	1.27646E+06	1.1333		1.71726E+07	0.27
Silanol, trimethyl-	TIC	2.5595	2.475	2.6083	1.93282E+07	1.1333		3.05032E+08	4.80
1,3-Butanediol	TIC	2.6225	2.6083	2.7083	1.70325E+06	1.1		1.21666E+07	1.29
Glycine	TIC	2.7347	2.7083	2.8083	1.61542E+06	1.1		1.91422E+07	0.30
Alanine, 3-amino-	TIC	2.8194	2.8083	2.8472	1.45247E+05	1.0389		1.98252E+06	0.06
Glycine	TIC	2.8558	2.8472	2.8861	1.36193E+05	1.0389		1.85714E+06	0.04
Disiloxane, hexamethyl-	TIC	2.9404	2.8861	2.9583	1.24407E+06	1.0722		1.69271E+06	0.15
Disiloxane, hexamethyl-	TIC	2.9766	2.9583	3.0028	1.19059E+05	1.0444		1.65778E+06	0.06
Disiloxane, hexamethyl-	TIC	3.0543	3.0028	3.175	5.89556E+07	1.1722		1.35271E+08	6.85
Butanal, 3-methyl-	TIC	3.2125	3.175	3.2583	1.07039E+07	1.0833		2.15644E+08	3.39
Butanal, 2-methyl-	TIC	3.2875	3.2583	3.3972	1.32868E+07	1.1389		1.45534E+08	2.29
2-Propanol, 1-amino-	TIC	3.4222	3.3972	3.4917	1.21993E+05	1.0944		1.19054E+06	0.08
Ethylenediamine	TIC	3.5142	3.4917	3.5417	1.16783E+05	1.05		1.51253E+05	0.01
2-Pentanone	TIC	3.5857	3.5417	3.6306	1.7061E+07	1.0889		1.5154E+07	1.34
Pentanal	TIC	3.6642	3.6306	3.7083	1.83102E+06	1.0778		1.14948E+07	0.50
Butanal, 3-hydroxy-	TIC	3.724	3.7083	3.7583	1.69842E+05	1.05		1.82874E+06	0.04
Butanal, 3-hydroxy-	TIC	3.7742	3.7583	3.7917	1.20725E+05	1.0333		1.57962E+05	0.02
Butanal, 3-hydroxy-	TIC	3.8155	3.7917	3.9083	1.87811E+05	1.1167		2.50239E+06	0.04
Cystine	TIC	3.9674	3.9361	3.9861	1.43167E+05	1.05		1.1786E+06	0.02
2-Oxa-1,3-disilacyclohexane, 1,1,3,3-tetramethyl-	TIC	4.0148	3.9861	4.0528	1.29845E+06	1.0667		1.83173E+07	0.29
Disulfide, dimethyl	TIC	4.1315	4.0528	4.1472	2.09893E+07	1.0945		1.30626E+07	1.46
Methyl Isobutyl Ketone	TIC	4.1706	4.1472	4.2361	1.96994E+07	1.0889		1.71717E+08	2.70
D-erythro-Pentose, 2-deoxy-	TIC	4.2462	4.2361	4.275	1.98584E+05	1.0389		1.99158E+06	0.06
Toluene	TIC	4.3084	4.275	4.3361	1.40034E+06	1.0611		1.74093E+07	0.27
2-Octene	TIC	4.3523	4.3361	4.3861	1.27163E+06	1.05		1.19802E+07	0.19
Octane	TIC	4.4099	4.3861	4.4472	1.68034E+06	1.0611		1.15005E+07	0.50
Butanal, 3-hydroxy-	TIC	4.4716	4.4472	4.4917	1.69554E+05	1.0444		1.26761E+06	0.02
Cyclotrisiloxane, hexamethyl-	TIC	4.5505	4.4917	4.6306	1.19364E+07	1.1389		2.45777E+08	3.87
2-Hexadecanol	TIC	4.6486	4.6306	4.675	1.97403E+05	1.0445		1.92554E+06	0.05
Pyrrole	TIC	4.7057	4.675	4.7472	1.51408E+06	1.0722		2.40744E+07	0.38
Propanoic acid, 2-methyl-	TIC	4.7685	4.7472	4.7806	1.26778E+06	1.0333		1.27265E+07	0.20
Hexanal	TIC	4.8129	4.7806	4.9417	1.26344E+07	1.1611		1.25459E+08	3.55



- *Nonanal,*
- *Decanal,*
- ***Butanoic acid,***
- ***Acetic acid***

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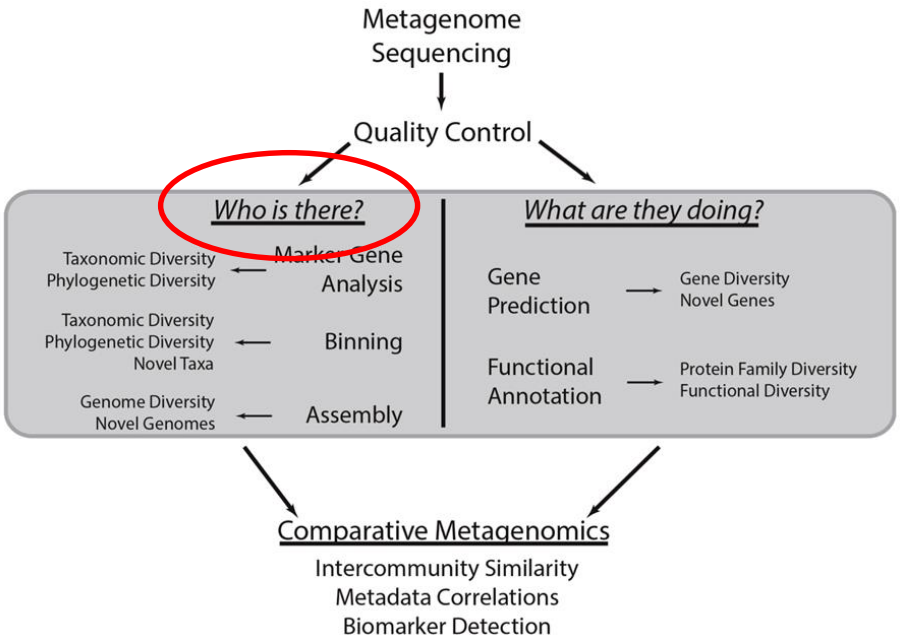
EXPLORING THE MICROBIOME

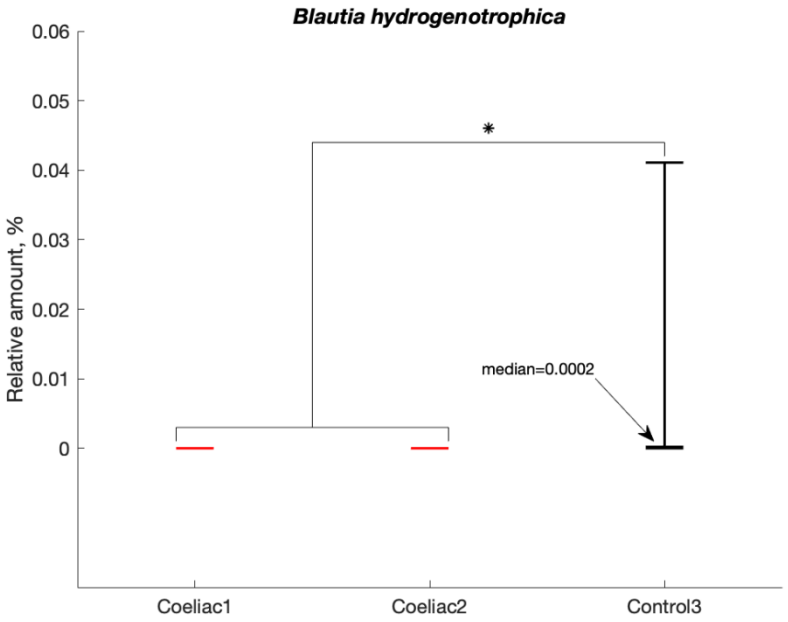
Microbiome – next generation sequencing

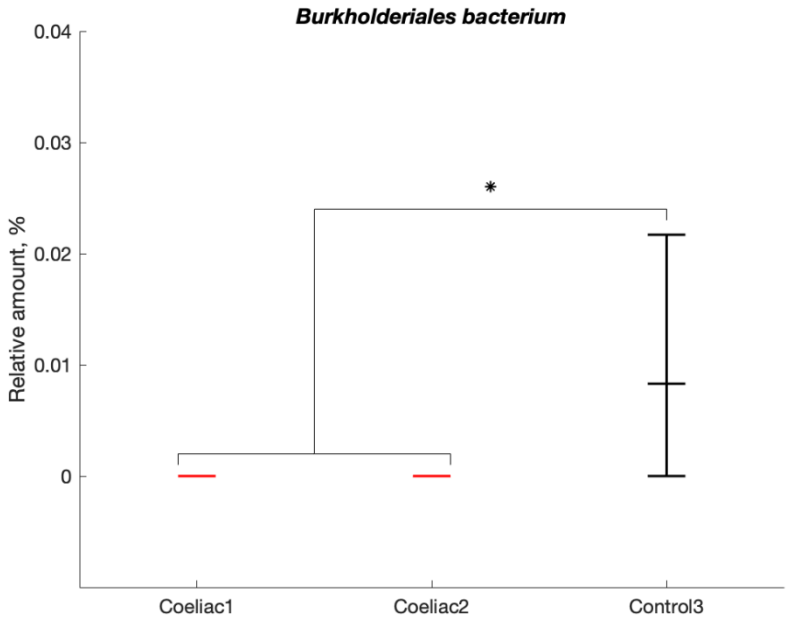
- shotgun metagenome sequencing (Nextseq 550)
- Entire genome within the sample
- Taxonomic classifier - to identify bacteria, archaea, virus and fungi communities in the samples.
- Relative abundance and diversity table
- Principle component analyses was used to identify differential features in that data

- Potential to explore functional pathways

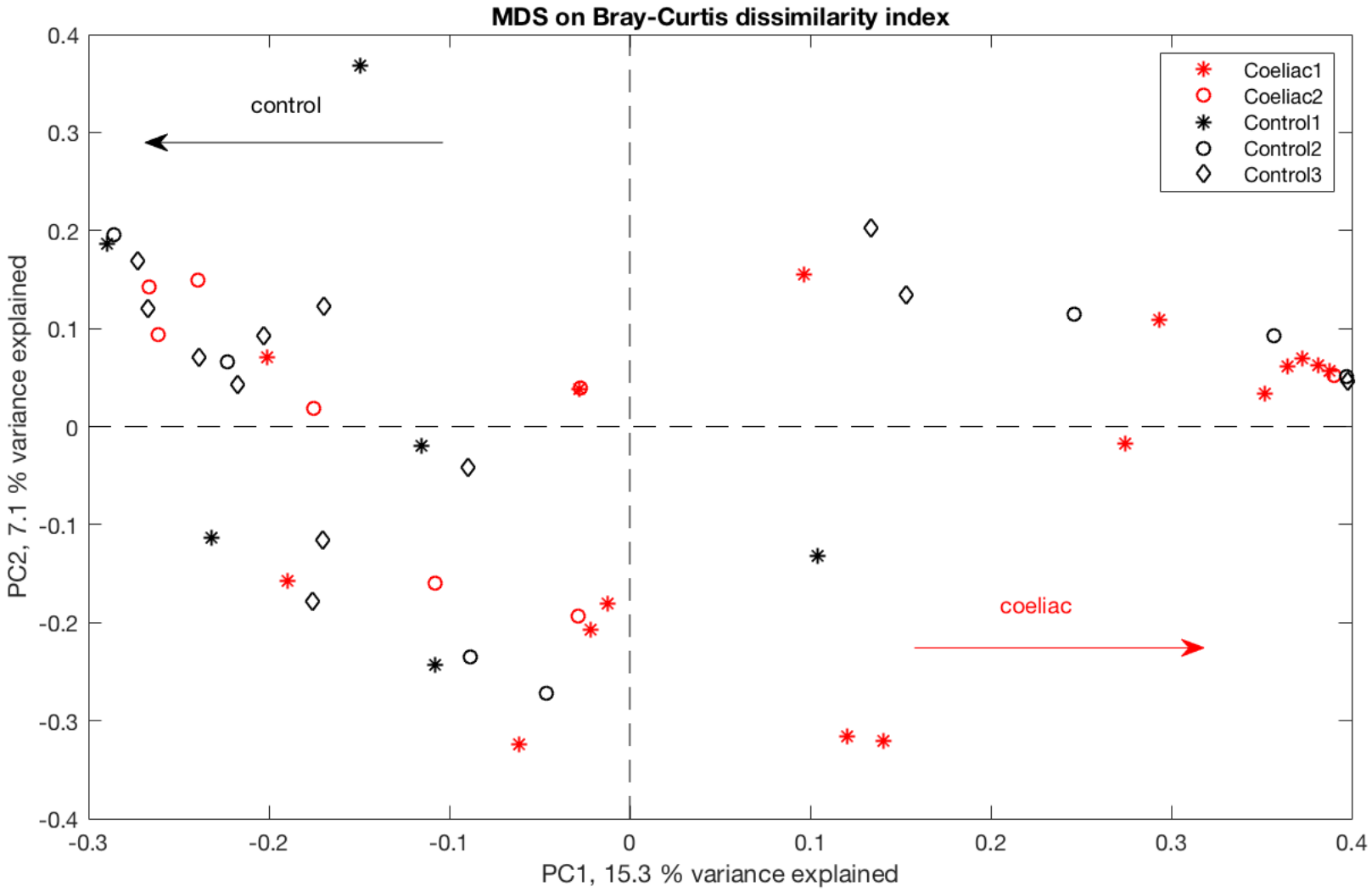


Bacteria - Firmicutes





Composition Index



Role of viruses?

- immune system of the intestinal mucosa can discriminate between pathogenic and commensal organism Li Z Plos One 2012
- Enterovirus possible trigger for CD Kahrs CR BMJ 2019
- Novel immune related genes (WES) Mistry I Plos One 2015
  - Exploratory work
  - Using the data from Mistry et al (2015), we selected two rare immune related genes (from their deep exosome study) to further analyse the presence of mutations in our samples

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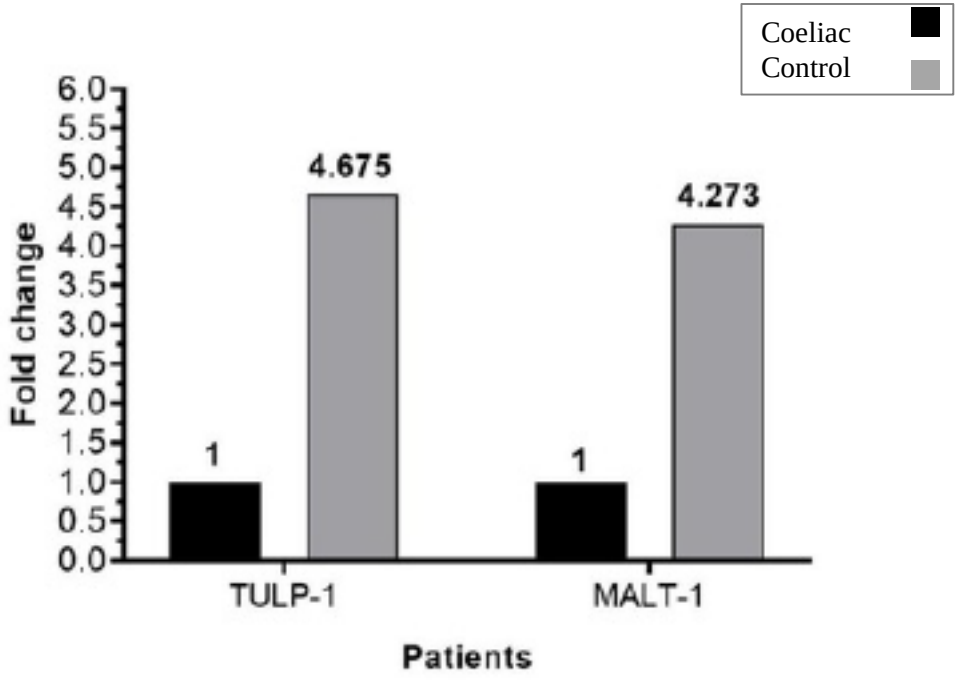
Genes selected where TULP1 and MALT1

Gene	Chr: position	SNV	PolyPhen Prediction	dbSNP132 ID/function	Cases validated/Cases tested
FAM179A	2:29259543	c.2555T>C	-	rs72788155/missense	2/6
*NLRC4**	2:32474767	c.2166T>G	Probably damaging	-	6/6
*EPAS1**	2:46607609	c.1798G>A	Possibly damaging	-	7/7
STON1	2:48809609	c.1837C>G	Probably damaging	-	2/7
*ARHGAP25**	2:69040504	c.739G>A	Probably damaging	rs61758703/missense	4/4
IQGAP2	5:75969341	c.3136G>T	-	-	1/6
DMGDH	5:78293933	c.2573A>C	Probably damaging	-	4/6
KIF13A	6:17826085	c.1700A>C	-	-	5/5
BRD2	6:32942277	c.68G>A	Probably damaging	rs55650502/missense	4/6
*GRM4**	6:34101193	c.81G>A	Benign	-	5/5
*TULP1**	6:35471412	c.1247G>A	Probably damaging	-	5/5
SYTL2	11:85445365	c.1004C>G	Probably damaging	rs74718633/missense	2/6
ABCA9	17:67039672	c.758C>T	Possibly damaging	-	4/4
*KCNJ16**	17:68129412	c.1184A>G	Benign	-	4/4
SDK2	17:71431712	c.1072C>T	-	-	1/4
*MALT1**	18:56402558	c.1567G>A	Probably damaging	-	6/6
*ACOT8**	20:44470575	c.862C>T	Probably damaging	-	4/4
EYA2	20:45808514	c.1267C>T	Possibly damaging	-	1/4

Reduced expression of both TULP1 & MALT-1 in those with CD



Conclusions

- Utility of urine VOCs to detect changes in those with coeliac before and after treatment
- Chemicals detected accompany observed changes in the microbiome – uncultured species; Discovery phase
- Microbiome shift towards controls in those with coeliac disease that commence a gluten free diet
  - Further exploration of immune related genes and viral triggers
  - specific chemicals and bacterial metabolic function (‘what are they doing?’)

Early insight into metabolic changes at cellular level in those with coeliac disease

Acknowledgements

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- Alfian Wicaksono & James Covington – School of Engineering, Warwick, UK
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- All our patients attending UHCW NHS Trust who participated

University of Oslo

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