Fecal water metabolomics in juvenile spondyloarthritis

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GBSC724 – Metabolomics
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Juvenile idiopathic arthritis

- Arthritis of unknown etiology starting in a child under age 16, lasting ≥ 6 weeks
- Prevalence of 1:1000
- Considered to be the leading acquired cause of childhood disability

Spondyloarthritis

- Type of pediatric and adult arthritis
  - Constitutes about 15% of JIA
- Distinctive demographic & clinical features
- High prevalence of gut inflammation
  - Inflammatory bowel disease (IBD): 5 – 10%
  - Subclinical intestinal inflammation: 67%

Diseases falling within spondyloarthritis

Adults
- IBD-associated arthritis
- Reactive arthritis
- Ankylosing spondylitis (AS)
- Psoriatic arthritis
- Undifferentiated SpA

And in children
- Enthesitis-related arthritis (ERA)
- +/- Juvenile psoriatic arthritis

Why study the microbiota in spondyloarthritis?

- Reactive arthritis: triggered by microbes
- Lessons from inflammatory bowel disease
  - Clinical and genetic overlap with SpA
  - Altered microbial populations
  - Response to microbiota-altering therapies
- Clues from SpA animal models

IBD and spondyloarthritis

- Clinical links
  - Arthritis in 25% of IBD patients
  - 2/3 of SpA have intestinal inflammation
  - Gut inflammation and arthritis track together
- Genetic links
  - IL23R
  - TNFSF15
  - STAT3

Lees, *Gut* 2011;60:1739
Fantini, *World Jrnl Gastroenter* 2009;15:2472

Microbiota in IBD

- Altered microbiota in multiple studies
- Response to microbiota manipulation
  - Probiotics (ulcerative colitis)
  - Antibiotics (Crohn's Disease >> colitis)
  - Fecal transplant
- Key flagellin targets identified in CD
  - Antibodies unique to CD
  - Diagnostic and prognostic information

Young, *The microbiota in inflammatory bowel disease*, in The Microbiome in Rheumatic Diseases and Infection (1st ed), Springer Publishing, 2018.

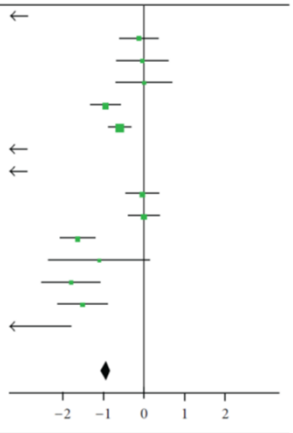
Microbiota in IBD

- Depletion of *Faecalibacterium prausnitzii* and other butyrate-producing organisms
- Depletion of *Bacteroides*
  - Consistent finding in adults only
- Enrichment for Enterobacteriaceae
  - Especially *Shigella* / Enteroinvasive *E. coli*

Decreased *F. praunitzii* in IBD

Study or subgroup	IBD (CD/UC) Mean	SD	Total	Healthy controls Mean	SD	Total	Weight	Std. mean difference IV, fixed, 95% CI
Andoh et al. 2012	0.4	0.09	67	0.81	0.04	121	3.4%	−6.53 [−7.26, −5.80]
Dörffel et al. 2012	9.06	9.33	50	10.21	9.94	25	7.9%	−0.12 [−0.60, 0.36]
Jia et al. 2010	5.71	5.34	20	5.93	5.83	18	4.5%	−0.04 [−0.68, 0.60]
Jia et al. 2010	5.93	5.87	14	5.93	5.83	18	3.7%	0.00 [−0.70, 0.70]
Joossens et al. 2011	9.44	1.85	68	10.97	1.25	55	12.9%	−0.94 [−1.32, −0.57]
Machiels et al. 2013	10.95	1.4	127	11.72	1.08	87	23.3%	−0.60 [−0.88, −0.32]
Sokol 2009	8.8	0.5	22	10.4	0.2	27	1.6%	−4.30 [−5.36, −3.25]
Sokol 2009	8.7	0.6	13	10.4	0.2	27	1.2%	−4.44 [−5.66, −3.22]
Swidsinski 2008	9.75	9.77	82	10.17	9.65	32	10.9%	−0.04 [−0.45, 0.37]
Swidsinski 2008	10.14	10	105	10.17	9.65	32	11.6%	−0.00 [−0.40, 0.39]
Varela 2013	8.02	0.57	116	8.9	0.37	31	9.4%	−1.64 [−2.08, −1.20]
Vermeiren 2011	5.56	0.83	6	6.63	0.95	6	1.2%	−1.11 [−2.36, 0.15]
Wang 2013	0.026	0.058	21	1.402	1.059	21	3.4%	−1.80 [−2.53, −1.07]
Wang 2013	0.225	0.512	34	1.402	1.059	21	4.7%	−1.52 [−2.13, −0.90]
Willing 2009	0.4	0.89	6	8.7	2.49	6	0.3%	−4.10 [−6.40, −1.80]
Total (95% CI)			**751**			**527**	**100.0%**	**−0.94 [−1.07, −0.80]**

Heterogeneity: $\chi^2 = 393.06$, df = 14 ($P < 0.00001$); $I^2 = 96\%$
Test for overall effect: $Z = 13.65$ ($P < 0.00001$)



Cao, *Gastro Enterol Res and Prac* 2014;2014:872725

F. prausnitzii is an anti-inflammatory organism

- Decreased fecal abundance in IBD[1]
- Increased IL-10 production by PBMCs[2,3]
- Reduces inflammation in colitis model[2]
- Major butyrate producer[4]
  - Beneficial effects on enterocytes[5]
  - Increases colonic regulatory T cells[6]

[1]Cao, *Gastroenterol Res Practice* 2014:872725 (review)
[2]Sokol, *PNAS* 2008;105:16731
[3]Rossi, *Sci Rep* 2016;6:18507
[4]Hold, *Appl Environ Microbiol* 2003;69:4320
[5]Hamer, *Aliment Pharmacol Ther 2008;27:104*
[6]Smith, *Science* 2013;341:569

Decreased *Bacteroides* in IBD

Abundance of *Bacteroides* in patients with IBD

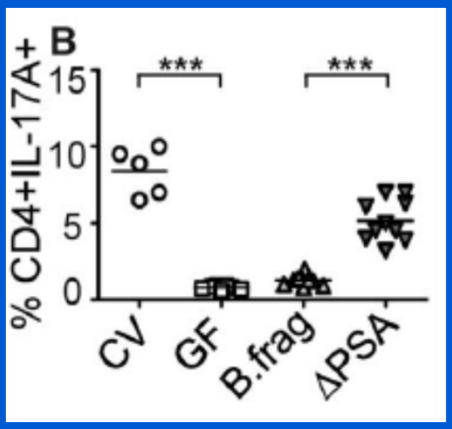
Metaanalysis of early (adult) studies

	Standard Mean Difference	95% CI	*P* value
CD versus control group	−1.42	(−1.94, −0.19)	$P < 0.001$
UC versus control group	−0.77	(−1.11, −0.42)	$P < 0.001$
CD versus UC	−0.38	(−0.85, 0.09)	0.12
**Active CD versus remission CD	−0.60	(−1.48, 0.28)	$P < 0.01$
***Active UC versus remission UC	−0.29	(−0.98, −0.09)	0.02

Zhou, *Biomed Res Int* 2016;2016:5828959

Polysaccharide A *on B. fragilis* inhibits development of Th17 cells

Colonic LP lymphocytes



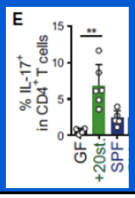
Round, *Science* 2011;332:974

Enteroinvasive *E. coli* and IBD

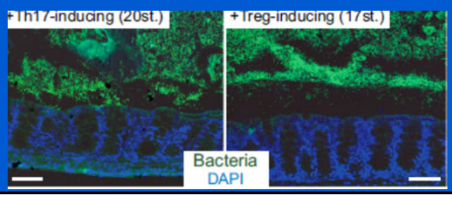
- Translocates across the intestinal barrier
  (Chassaing, *J Clin Invest* 2011;121:966)
- α-hemolysins damage intestinal barrier
  (Bucker, Gut 2014;63:1893)
- Maturation of Th17 cells

Adhesive bacteria induce Th17 cells

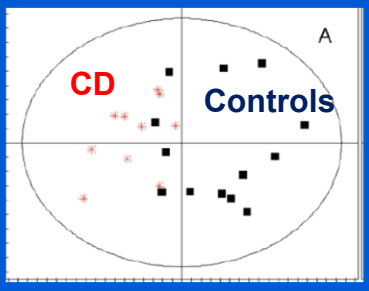
- Ampicillin-treated microbiota from UC patients induced Th17 cells in GF mice
- 20 species were cultured and collectively inoculated into GF mice
- Results
  - Induced Th17 cells
  - Upregulated Nos2 (marker of adhesion)
  - Adhesion directly observed

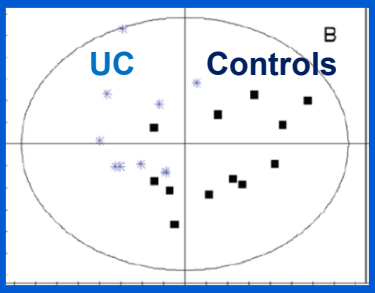


Atarashi, *Cell* 2015;163:367



Fecal metabolomics in IBD

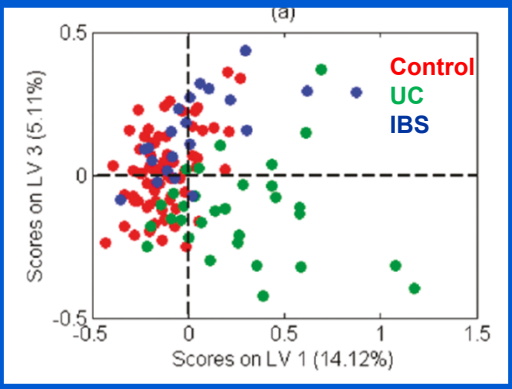


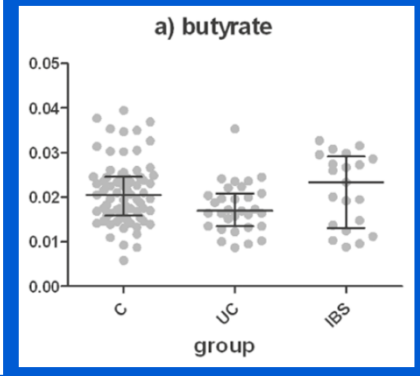


metabolite	CD vs Control	UC vs Control	CD vs UC
	$R^2 = 0.92$	$R^2 = 0.77$	$R^2 = 0.93$
	$Q^2 = 0.80$	$Q^2 = 0.52$	$Q^2 = 0.70$
acetate	−0.67		−0.50
alanine	+0.64		+0.72
butyrate	−0.64	−0.36[b]	
glutamate		+0.68	
glycerol			+0.82
isoleucine	+0.69		+0.66
leucine	+0.74		+0.74
lysine	+0.72	+0.62	+0.67
methylamine	−0.73	−0.39	
trimethylamine	−0.52	−0.35	
unknown (1.33)	−0.82	−0.69	
valine	+0.66		+0.71

Marchesi, *J Proteome Res* 2007;6:546

Decreased fecal butyrate in IBD





Le Gall, *J Proteome Res* 2011;10:4208

Gut microbes in animal models of SpA

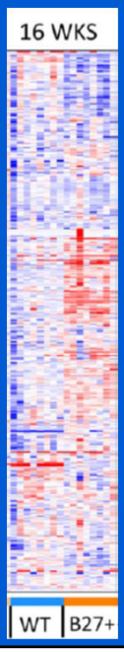
Feature	Rat model[1]	Mouse model[2]
Background	33-3 line	B10.BR
Transgene	HLA-B27, β2m	HLA-B27, β2m
Clinical features	Colitis, arthritis, spondylitis	Enthesitis, ankylosis of ankles and tarsal joints
Germ free state	Decreased colitis, arthritis	No disease
Germ free with defined bacteria	DESEP – No DESEP-B – Yes	Lactobacillus – no Mixture of 10 anaerobes – Yes

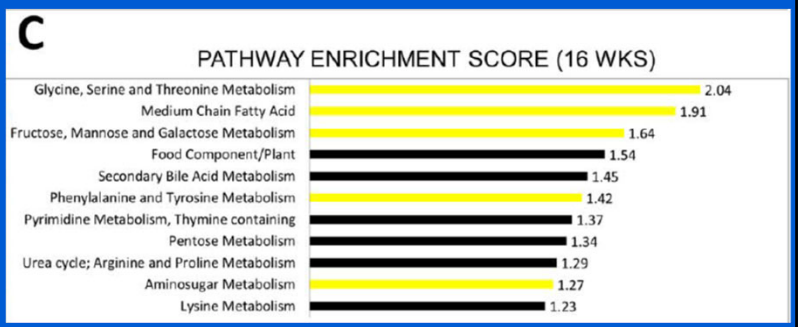
DESEP
Streptococcus faecum
Escherichia coli
Streptococcus avium
Eubacterium contortum
Peptostreptococcus productus

[1]Rath, *Jrnl Clin Invest* 1996;98:945
[2]Sinkorova, *Human Immunol* 2008;69:845

DESEP-B: DESEP + Bacteroides vulgatus

Metabolome of HLA-B27-tg rats

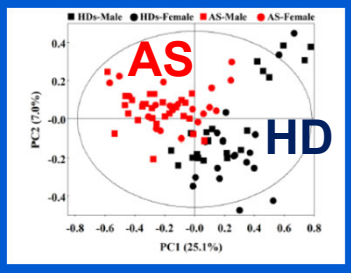


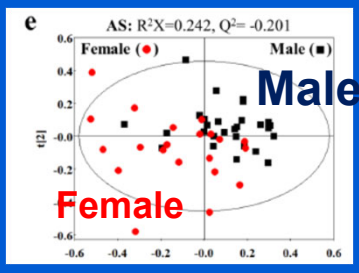


Asquith, *Arth Rheum* 2017;69:1984

Fecal metabolome in AS

Analysis limited to 84 (out of 2342) identifiable features





AS patients only

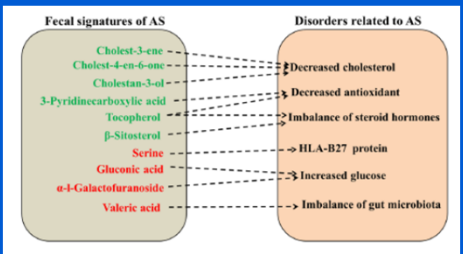
He, *Scientific Rep* 2019;9:3872

Fecal metabolome in AS

AS vs Controls

	Total—AS *vs* HD		
	VIP	P value	Trend
5-Trimethylsilyloxy-n-valeric acid	1.24	0.000	↑
Cyclohexanecarboxylic acid	3.27	0.000	↑
Cholestan-3-ol	3.92	0.001	↓
Tocopherol	2.73	0.001	↓
Gluconic acid	1.34	0.000	↑
β-Sitosterol	2.43	0.000	↓
Serine	1.37	0.001	↑
Stigmastan-3,5-diene	2.27	0.012	↓
24-Ethyl-δ(22)-coprostenol	1.55	0.000	↓
α-l-Galactofuranoside	1.35	0.000	↑
N-(4,5-Dimethyl-thiophen-2-yl)-benzamide	1.10	0.000	↑
3-Pyridinecarboxylic acid	1.54	0.000	↓
Cholest-3-ene	1.19	0.007	↓
Docosanoic acid	1.71	0.043	↑
Cholest-4-en-6-one	1.49	0.000	↓
1-Heptatriacotanol	1.07	0.000	↓
Nonacosane	1.37	0.003	↑
Ergost-5-en-3-ol, acetate	<1.00	0.007	—
D-Myo-Inositol	<1.00	0.010	—

Pathways



He, *Scientific Rep* 2019;9:3872

Fecal metabolome in AS

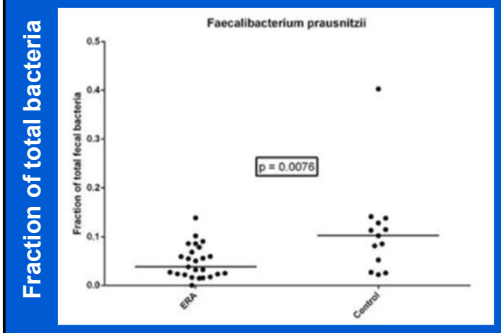
Male vs female

	Total — Male *vs* female			AS — Male *vs* female		
	VIP	P value	Trend	VIP	P value	Trend
α-Tocopherol	2.46	>0.05	—	3.47	>0.05	—
Docosanoic acid	3.07	>0.05	—	3.38	>0.05	—
Tocopherol	3.74	>0.05	—	3.37	>0.05	—
11-cis-Octadecenoic acid	2.14	>0.05	—	2.36	>0.05	—
α-l-Galactofuranoside	1.94	>0.05	—	2.31	>0.05	—
Cholestan-3-ol	2.80	>0.05	—	2.18	>0.05	—
Cyclohexanecarboxylic acid	2.15	>0.05	—	2.07	>0.05	—
Stigmastan-3,5-diene	2.87	>0.05	—	1.81	>0.05	—
Cholest-4-en-6-one	1.05	>0.05	—	1.75	0.041	↓
α-D-Glucopyranoside	1.54	>0.05	—	1.51	>0.05	—
Nonacosane	1.63	>0.05	—	1.29	>0.05	—
Serine	1.30	>0.05	—	1.27	>0.05	—
Cholest-3-ene	<1.00	>0.05	—	1.14	>0.05	—
Propanedioic acid	<1.00	>0.05	—	1.12	0.020	↑
5-Hydroxyhexanoic acid diTMS	<1.00	>0.05	—	<1.0	0.039	—
D-Myo-Inositol	<1.00	>0.05	—	<1.0	0.045	—
Pentacosane	<1.00	>0.05	—	<1.0	0.038	—
β-Sitosterol	1.06	>0.05	—	<<1.00	>0.05	—
Carbazole	<1.00	>0.05	—	<1.00	>0.05	—
Galactose oxime	<1.00	>0.05	—	<1.00	>0.05	—
9,12-Octadecadienoic acid	<1.00	>0.05	—	<1.00	>0.05	—
Ergost-5-en-3-ol, acetate	<1.00	>0.05	—	<1.00	>0.05	—
d-Glucose	1.14	>0.05	—	<1.00	>0.05	—
Acetic acid	1.14	>0.05	—	<1.00	>0.05	—
9-Octadecenoic acid	<1.00	>0.05	—	<1.00	>0.05	—

He, *Scientific Rep* 2019;9:3872

Microbiota in JSpA (ERA); 2014

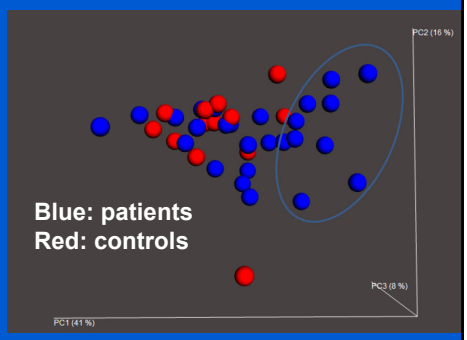
Fecal abundance of *F. prausnitzii*



Fraction of total bacteria

ERA Controls

Principal coordinates analysis



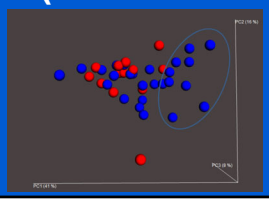
Blue: patients
Red: controls

Stoll, *Arth Res Ther* 2014;16:486

Clusters are defined by *Bacteroides* and *Akkermansia*

- The two clusters had similar abundance of *F. Prausnitzii*
- The subjects who clustered with the controls tended to have high abundance of *Akkermansia* (> 1% in 7/17 vs 0/8)
- The subjects forming their own cluster had high *Bacteroides* abundance (32% vs 13%)

Stoll, *Arth Res Ther* 2014;16:486

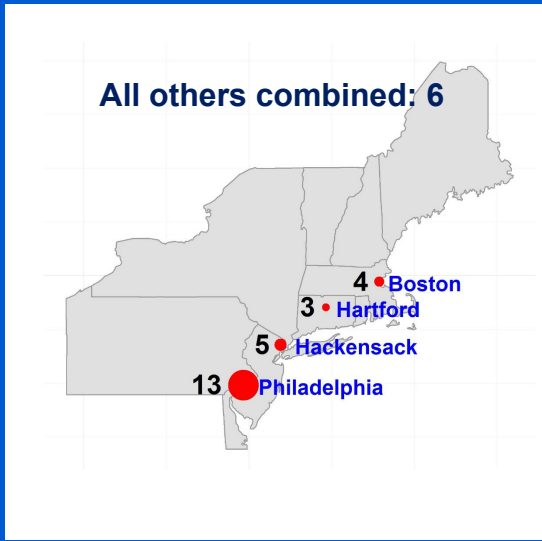


What about the impacts of treatment?

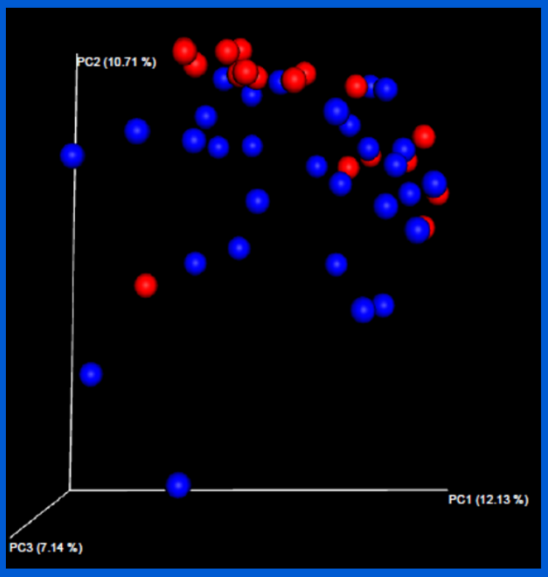
Studied a multi-center cohort of newly diagnosed patients

Collaborating centers

Collaborators are not all created equal



Clustering by diagnosis

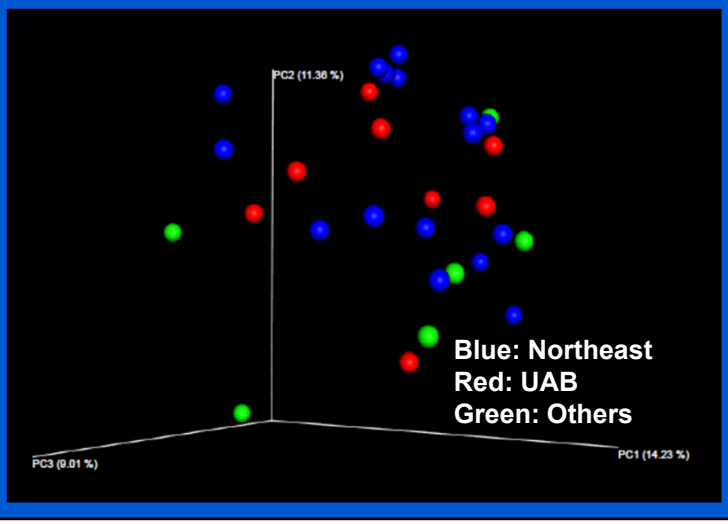


Blue: ERA
Red: Control

Stoll, *Arth Res Ther* 2018;20:14

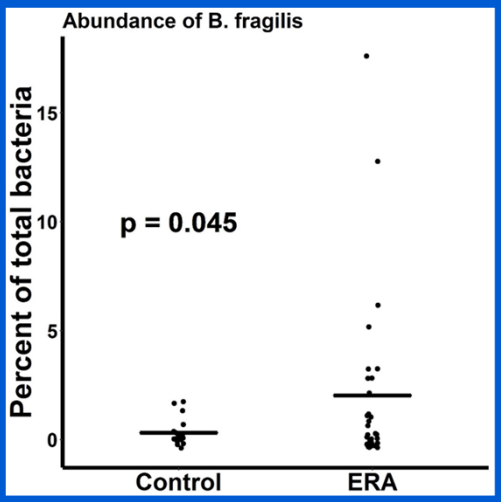
But not by geography

Limited to ERA patients



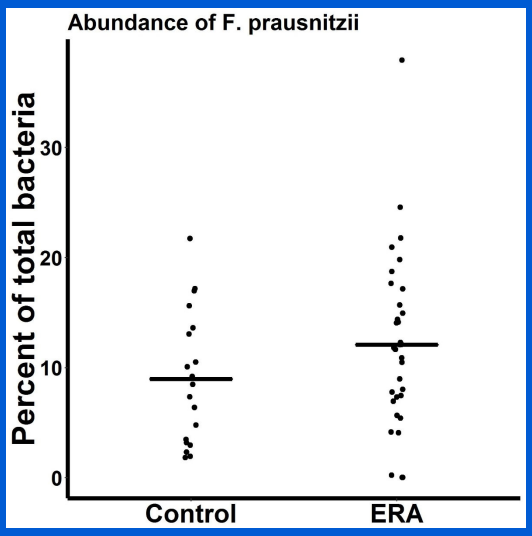
Blue: Northeast
Red: UAB
Green: Others

Increased *B. fragilis* in ERA



Stoll, *Arth Res Ther* 2018;20:14

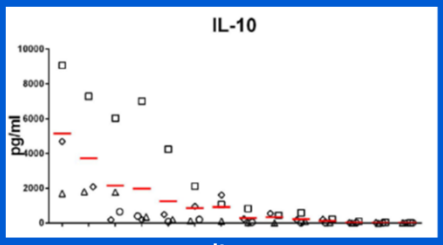
No overall differences in *F. praunitzii*



Stoll, *Arth Res Ther* 2018;20:14

There are differences within strains of *F. prausnitzii*

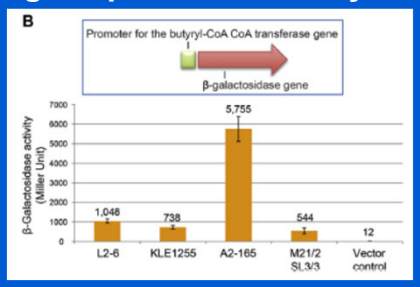
IL-10 production from hDC



A2-165 L2/6 M21/2 S3I/3 HTF-F

Rossi, *Sci Rep* 2016;6:18507

Butyryl-CoA transferase gene promoter activity

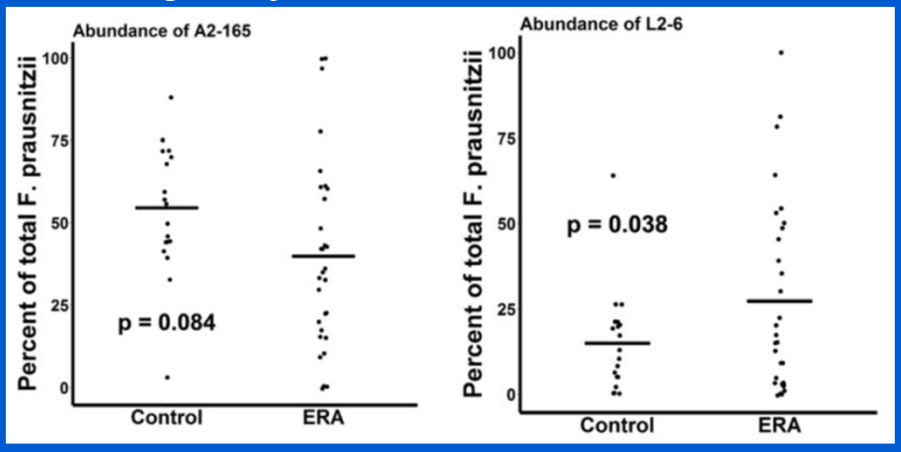


L2/6 KLE1255 A2-165 M21/2 Vector control

Song, *JACI* 2016;137:852

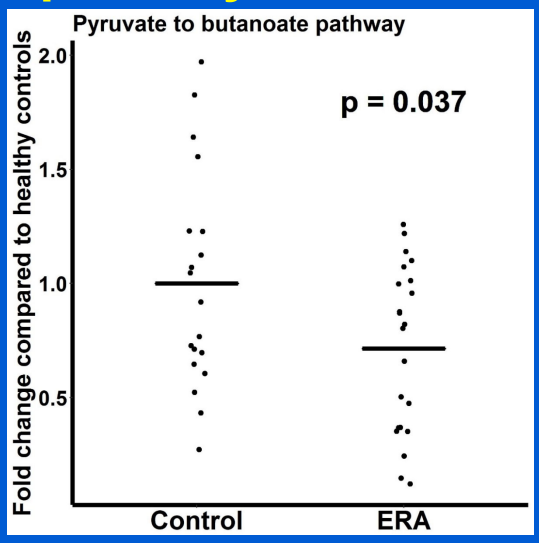
Decreased regulatory strain of *F. prausnitzii* in ERA

Regulatory strain **Control strain**



Stoll, *Arth Res Ther* 2018;20:14

Lower coverage of the butanoate pathway in ERA

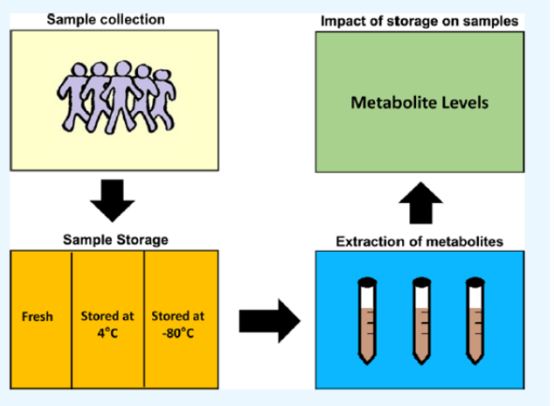


Stoll, *Arth Res Ther* 2018;20:14

Are these taxonomic and genetic alterations associated with different metabolic products?

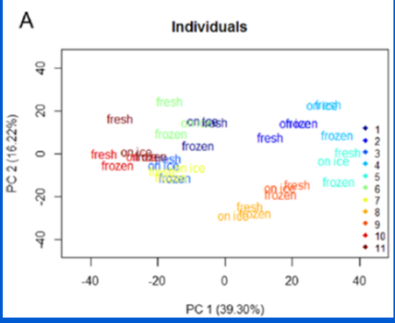
To be addressed with nanoLC-MS

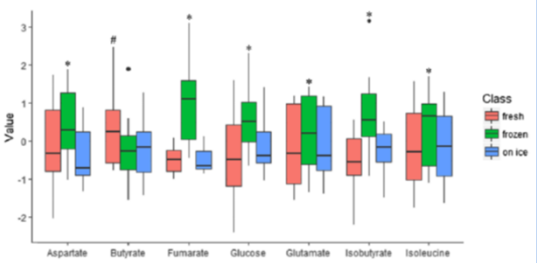
First question: Does -80oC storage impact findings?



O'Sullivan, *ACS Omega* 2018;3:16585

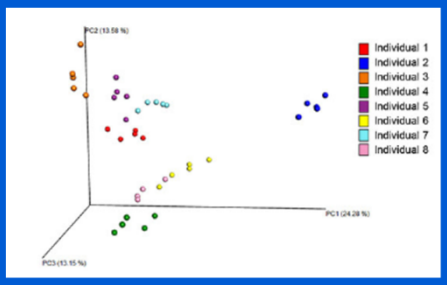
Looks like it does not much

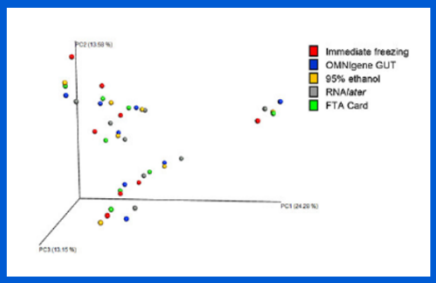




O'Sullivan, *ACS Omega* 2018;3:16585

Nor does the collection technique

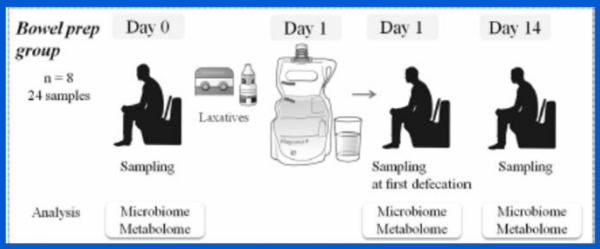


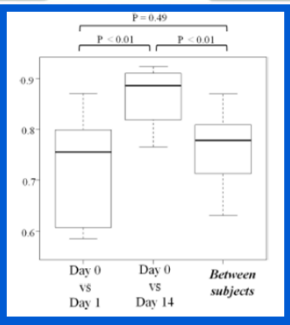


Except that RNALater and metabolomics do not agree with one another

Wang, *Front Cell Infec Microbiol* 2018;8:301

What about the bowel prep?





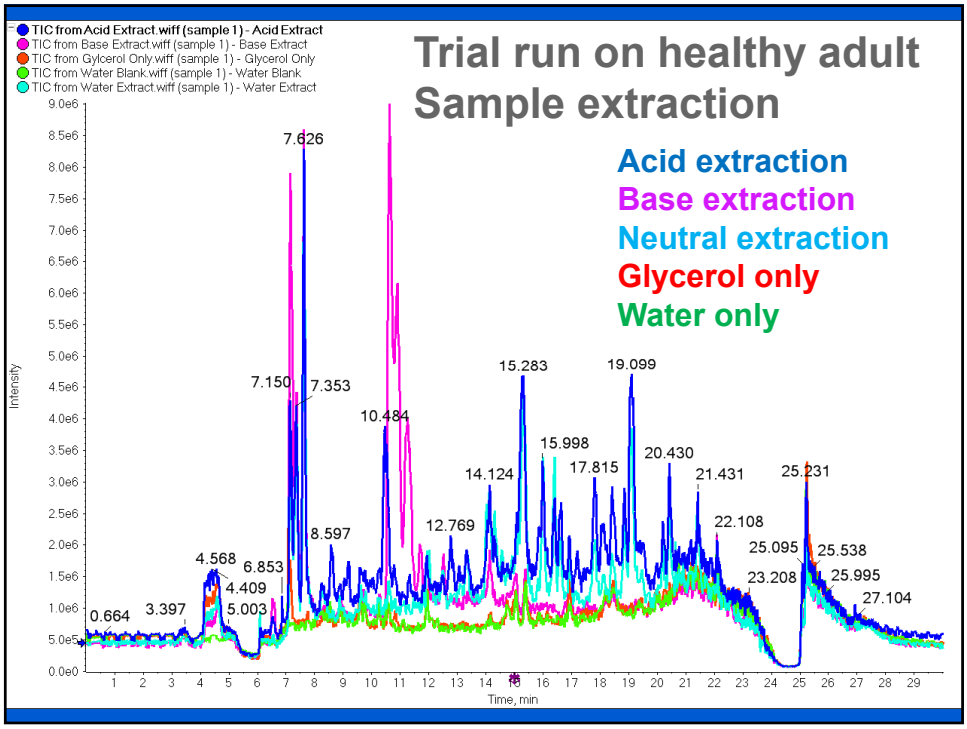
Nagata, *Scientific Rep* 2019;9:4042

Next questions

- How to prepare the fecal specimen?
- Will the glycerol impact the findings?
  - Will it destroy the machine? (Bad outcome)

Sample collection

- Samples collected in Cary-Blair media and stored at -80°C in glycerol
- ddH20 added 1:1 to thawed suspension
- Addition of
  - Acid: 1 µL / ml of 98% formic acid
  - Alkaline: 90 µL of 0.15M NaOH
  - Neutral: Nothing added
- Ultracentrifuge (14,000g x 15min at 4oC)
- Extraction with ethyl acetate

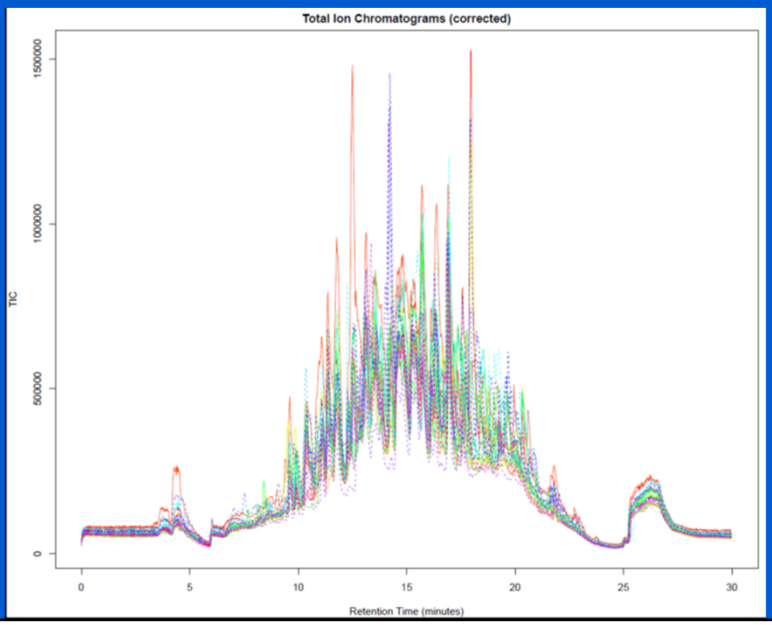


Metabolomics in ERA

- Obtained fecal water specimens from two cohorts of children with ERA
- Excluded use of antibiotics within 3 months
- Subjected to nanoLC-MS
- Acid extraction

Feature	Derivation set		Validation set	
	ERA	Control	ERA	Control
N	14	9	10	10
Age (yrs)*	14; 7-17	10; 7-18	14; 8-16	12; 9-17
Male : female	5 : 9	2 : 7	7 : 3	5 : 5
BMI*	26; 17-35	19; 14-24	20; 15-27	19; 15-32
HLA-B27+	2 / 13	ND	4 / 10	ND
Duration of therapy (months)	0; 0-2	NA	4; 0-27	NA
Meds				
None	5	11	0	11
MTX alone	6	0	3	0
MTX, anti-TNF	2	0	4	0
Anti-TNF alone	1	0	3	0

Total Ion Chromatogram



Output

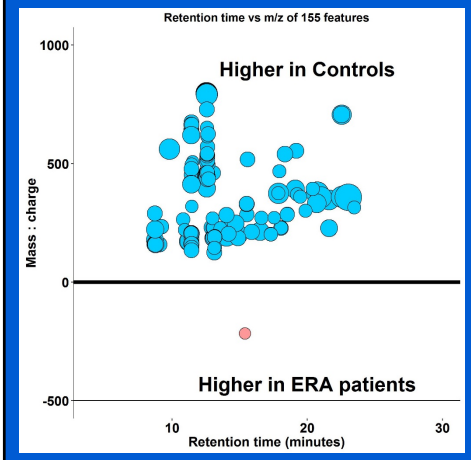
	name	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	npeaks	maxint	mean1	sd1	mean2	sd2	AIM10
1	M375T11	375.1196	375.1157	375.1242	10.518	10.4453	10.66235	9	1489	11612.01	1988.292	8031.63	2258.383	13632.6
2	M145T17	145.0832	145.0751	145.0854	16.5405	16.50928	16.57477	25	6412	31653.72	5460.644	40894.85	8253.572	28153.01
3	M230T13	230.142	230.1357	230.1455	12.92336	12.77142	13.03595	22	5038	44762.31	17914.8	23288.23	11603.87	71505.83
4	M217T15	217.0594	217.0552	217.0629	15.3946	15.34805	15.4613	17	2467	12993.46	3934.568	18000.05	4133.412	11646.65
5	M250T23	250.1544	250.1532	250.1597	22.90225	22.83	22.9265	16	146	528.6872	114.1793	708.1843	187.559	429.0455
6	M171T5	170.9971	170.9959	171.0015	4.796133	4.6058	5.027267	24	1018	12134.58	2157.723	16006.23	4290.593	9046.578
7	M541T18	541.3654	541.3644	541.3718	18.33025	18.2465	18.382	8	610	6100.533	2595.559	3432.371	1029.915	7482.783
8	M554T19	554.3772	554.3712	554.3797	19.17725	19.05517	19.2615	18	828	7444.75	3638.92	3894.982	843.9107	10250.25
9	M540T18	540.3623	540.3603	540.3713	18.34017	18.31633	18.37383	15	1886	11959.26	6837.767	5657.515	1965.369	16554.72
10	M392T19	392.2578	392.2512	392.2625	19.11833	19.088	19.16567	17	1478	10284.84	7512.577	3370.939	2008.293	17069.17
11	M458T13_	458.226	458.2184	458.2302	13.06881	13.0053	13.13973	8	2050	13824.57	4209.204	9867.679	2206.84	18814.28
12	M517T16	517.2108	517.2061	517.2152	15.57198	15.53338	15.6045	11	6326	26653.93	14532.22	13350.45	7289.252	22128
13	M374T18	374.254	374.2489	374.2614	17.8755	17.83267	17.91267	13	2809	20887.82	18428	4637.943	2698.356	41094.56
14	M346T22	346.2578	346.2563	346.2582	21.61733	21.55183	21.83467	8	566	2918.221	2526.624	707.5931	472.1762	6226.067

Initial data processing

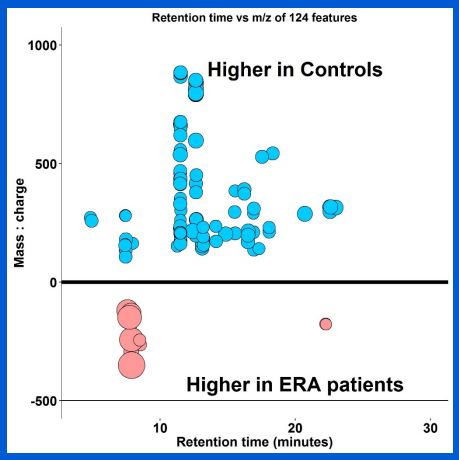
- **Pairwise comparisons for each ion**
  - Comparing patients to controls
- **Created two lists of ions with fold differences of ≥ 1.5, p-values ≤ 0.05**
  - One list: ions higher in patients
  - Other list: ions higher in controls

Fewer unique metabolites in ERA

Negatively charged ions

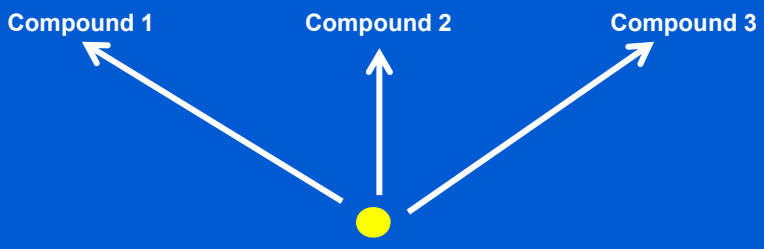


Positively charged ions



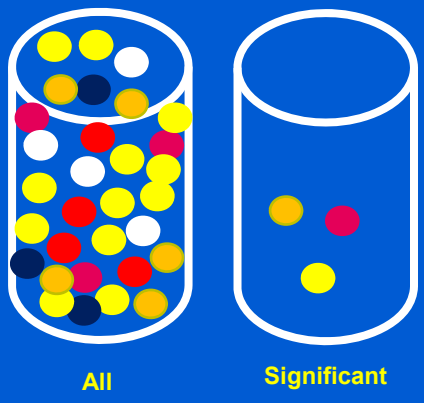
Stoll *Genes Immunity* 2016;17:400

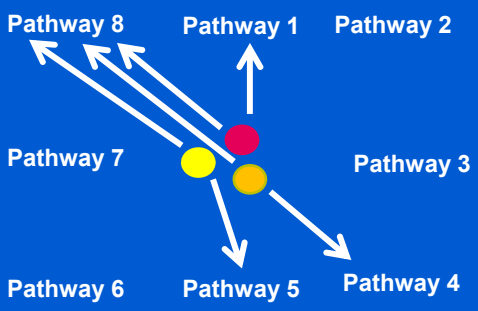
Identifying the ions: the challenge



One ion (identified by m/z) can represent multiple different compounds

Identifying the ions: mummichog





Li, *PLoS Comput Biol* 2013;9:e1003123
http://mummichog.org/index.html

Using mummichog

- Download mummichog, dependencies
- Need 2 input files (.txt files, no column headings, cols are m:z,RT,p-value,t-stat)
  - Reference file: full list of ions
  - Input file: partial list
- mummichog/main.py -r REFERENCE -i INPUT -o OUTPUT -m positive [negative]

http://mummichog.org/

Mummichog output

- Folder with three sub-folders, txt file, and html file
- HTML file shows top pathways from the input file
- TXT file is a log file
- TSV folder contains useful datafiles

Tentative feature match

m/z	id	match_forr	mz_differe	name	pathway
63.9942	C00084	M+Na-2H[-	0.0006	Acetaldehy	Glycine, serine, alanine and threonine metabc
63.9942	C06548	M+Na-2H[-	0.0006	Ethylene oxide	
96.9608	C00059	M-H[-]	0.0007	Sulfate; Sul	Glycosphingolipid metabolism$Androgen and
96.9608	C00094	M-H+O[-]	0.0007	Sulfite	Methionine and cysteine metabolism
105.0194	C00033	M+HCOO[-	0.0007	Acetate; Ac	Pyruvate Metabolism$Proteoglycan biosynthe
105.0194	C00058	M+CH3CO(	0.0006	Formate; N	Squalene and cholesterol biosynthesis$Tryptc
105.0194	C00184	M-H+O[-]	0.0001	Glycerone;	Glycerophospholipid metabolism
105.0194	C00186	M-H+O[-]	0.0001	(S)-Lactate;	Pyruvate Metabolism$Glycolysis and Glucone
105.0194	C00256	M-H+O[-]	0.0001	(R)-Lactate	Pyruvate Metabolism$Glycine, serine, alanine
105.0194	C00258	M-H[-]	0.0001	D-Glycerat	Glycine, serine, alanine and threonine metabc
105.0194	C00266	M+HCOO[-	0.0007	Glycolaldeł	Glyoxylate and Dicarboxylate Metabolism
105.0194	C00577	M-H+O[-]	0.0001	D-Glyceral	Galactose metabolism$Glycerophospholipid r
105.0194	C01013	M-H+O[-]	0.0001	3-Hydroxy	Beta-Alanine metabolism$Propanoate metab
105.0195	C00033	M+HCOO[-	0.0008	Acetate; Ac	Pyruvate Metabolism$Proteoglycan biosynthe
105.0195	C00058	M+CH3CO(	0.0007	Formate; N	Squalene and cholesterol biosynthesis$Tryptc
105.0195	C00184	M-H+O[-]	0.0002	Glycerone;	Glycerophospholipid metabolism
105.0195	C00186	M-H+O[-]	0.0002	(S)-Lactate;	Pyruvate Metabolism$Glycolysis and Glucone
105.0195	C00256	M-H+O[-]	0.0002	(R)-Lactate	Pyruvate Metabolism$Glycine, serine, alanine
105.0195	C00258	M-H[-]	0.0002	D-Glycerat	Glycine, serine, alanine and threonine metabc
105.0195	C00266	M+HCOO[-	0.0008	Glycolaldeł	Glyoxylate and Dicarboxylate Metabolism
105.0195	C00577	M-H+O[-]	0.0002	D-Glyceral	Galactose metabolism$Glycerophospholipid r
105.0195	C01013	M-H+O[-]	0.0002	3-Hydroxy	Beta-Alanine metabolism$Propanoate metab
116.0718	C00183	M-H[-]	0.0001	L-Valine; 2-	Valine, leucine and isoleucine degradation

Pathways represented in controls

Pathway	Overlap size	Pathway size	Corrected p-value
NEGATIVELY CHARGED IONS (top 5)			
Glycosphingolipid biosynthesis - ganglioseries	5	7	0.00091
Tryptophan metabolism	13	46	0.00106
Glycosphingolipid biosynthesis - globoseries	3	3	0.00122
Glycosphingolipid metabolism	6	15	0.00125
N-Glycan biosynthesis	3	6	0.00328
POSITIVELY CHARGED IONS (top 5)			
Tryptophan metabolism	9	37	0.0038
Xenobiotics metabolism	8	36	0.00544
Selenoamino acid metabolism	3	12	0.02442
Vitamin B6 (pyridoxine) metabolism	2	6	0.04015
Purine metabolism	4	24	0.05128

Stoll *Genes Immunity* 2016;17:400

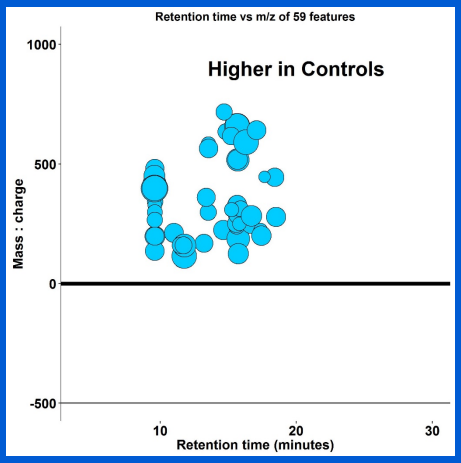
More data from the negative mode

Pathway	Overlap size	Pathway size	Corrected p-value
Glycosphingolipid biosynthesis - ganglioseries	5	7	0.00091
Tryptophan metabolism	13	46	0.00106
Glycosphingolipid biosynthesis - globoseries	3	3	0.00122
Glycosphingolipid metabolism	6	15	0.00125
N-Glycan biosynthesis	3	6	0.00328
Tyrosine metabolism	14	68	0.00349
Glycolysis and gluconeogenesis	6	23	0.00425
Butanoate pathway	4	12	0.00431
Biopterin metabolism	4	13	0.00431
Fructose and mannose metabolism	4	16	0.01275

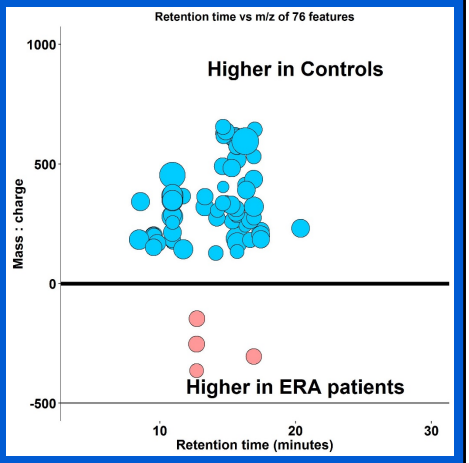
Stoll *Genes Immunity* 2016;17:400

Validation run

Negatively charged ions



Positively charged ions



Stoll *Genes Immunity* 2016;17:400

Pathways represented in controls

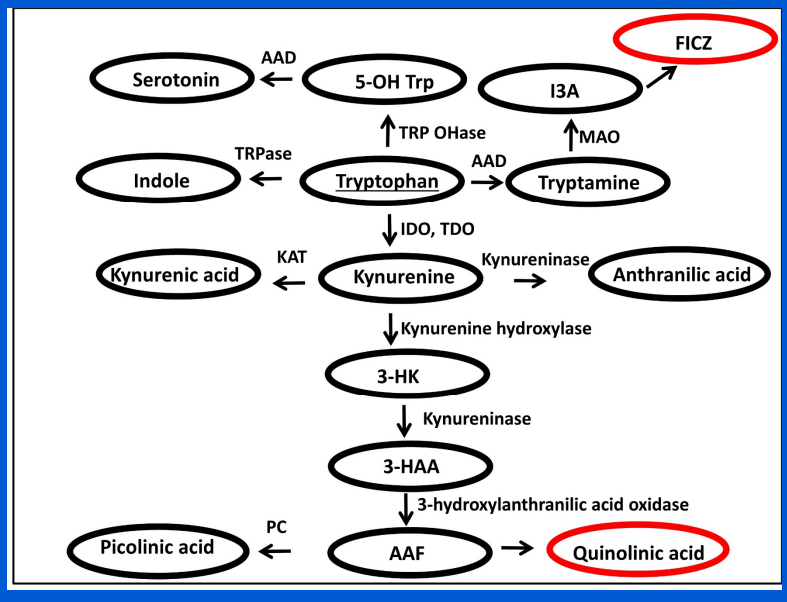
Pathway	Overlap size	Pathway size	Corrected p-value
NEGATIVELY CHARGED IONS			
Urea cycle/amino group metabolism	3	21	0.0106
Biopterin metabolism	2	7	0.01162
Tryptophan metabolism	3	37	0.03769
Glycerophospholipid metabolism	2	16	0.03784

Stoll *Genes Immunity* 2016;17:400

Tryptophan and SpA

- TRP is an essential amino acid
- Only about 1% of Trp is used for protein synthesis; rest is metabolized
- Most of the metabolites can impact systemic immunity
  - Th17 / regulatory T cell balance
  - Chemokines / cytokines
- Most, not all, are anti-inflammatory

Trp metabolic pathways



TRP and inflammatory disease

- Reduced plasma TRP in adults with ankylosing spondylitis (Gao, *Analyst* 2008; 133:1214)
- Reduced serum TRP in adults with IBD
  - Negative association with disease activity (Nikolaus, *Gastroenterology* 2017;153:1504)

Validation with targeted metabolomics

- Worked with metabolomics group to develop assays for:
  - TRP and relevant metabolites
  - SCFA
- Selected TRP metabolites with literature to suggest a role in arthritis or immune activation
- Newly diagnosed treatment naïve patients
  - To the extent possible, used patients from prior run
- Serum and stool

Selected metabolites

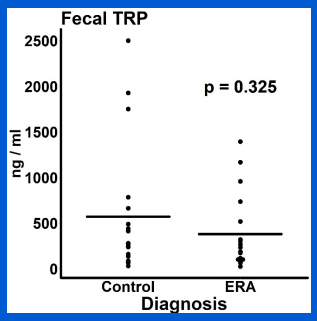
Tryptophan pathway
Tryptophan
Tryptamine
Nicotinic acid
Serotonin
2-picolinic acid
5-OH tryptophan
Indole-3 acetate
Indole-3 lactate
Kynurenine

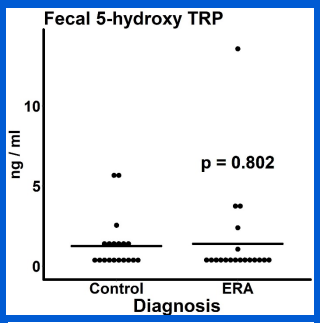
SCFA
Butyric acid
Acetic acid
Propionic acid
Valeric acid

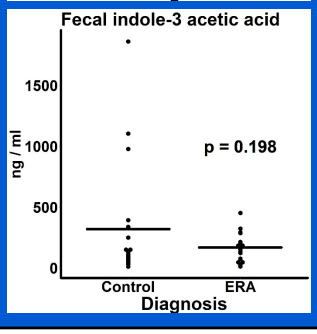
Subjects

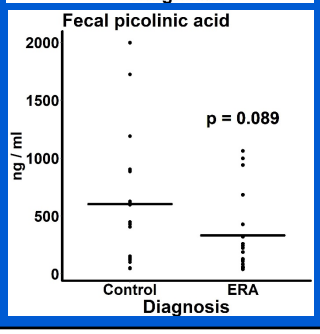
Characteristics	Stool	Serum
n	38	50
ERA : healthy control	19 : 19	25 : 25
Included in prior publication	11	13

Similar fecal TRP metabolites

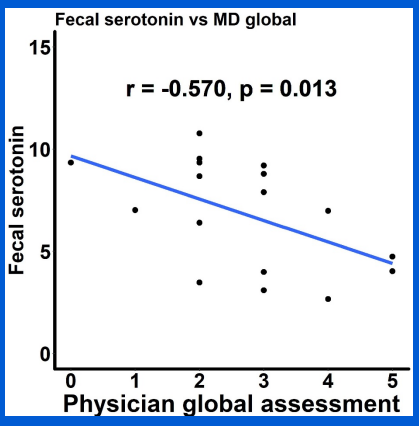


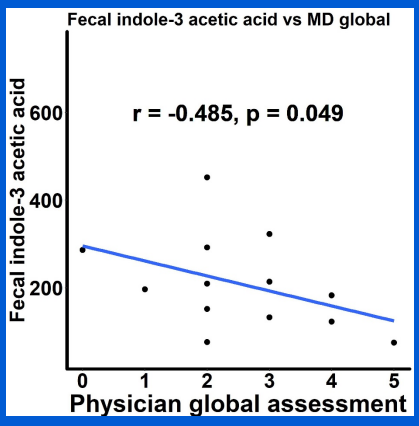




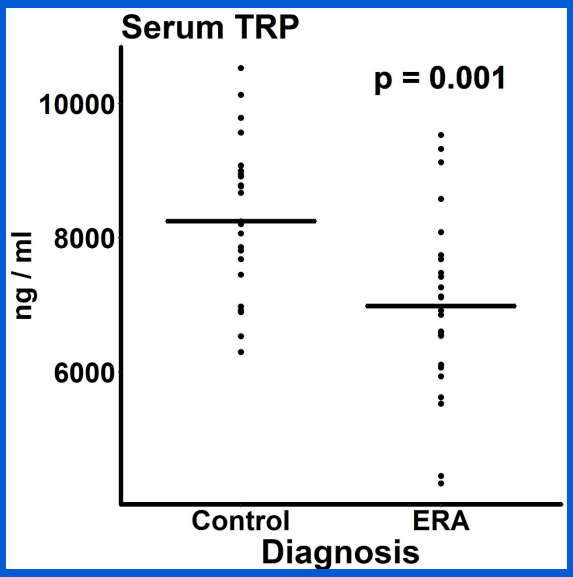


Some metabolites were inversely associated with disease activity among patients

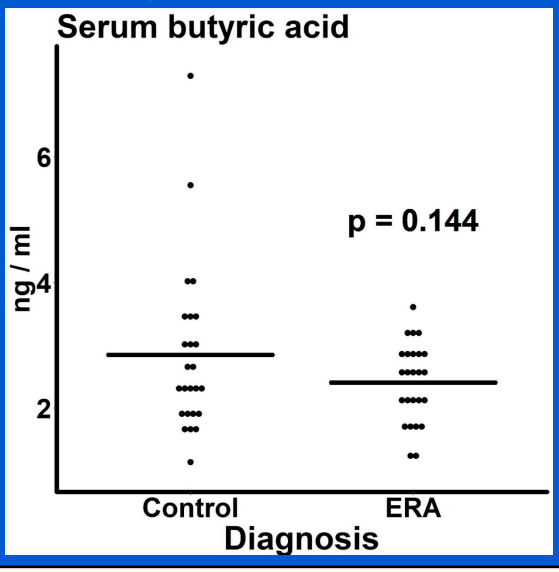




Decreased Serum TRP in ERA

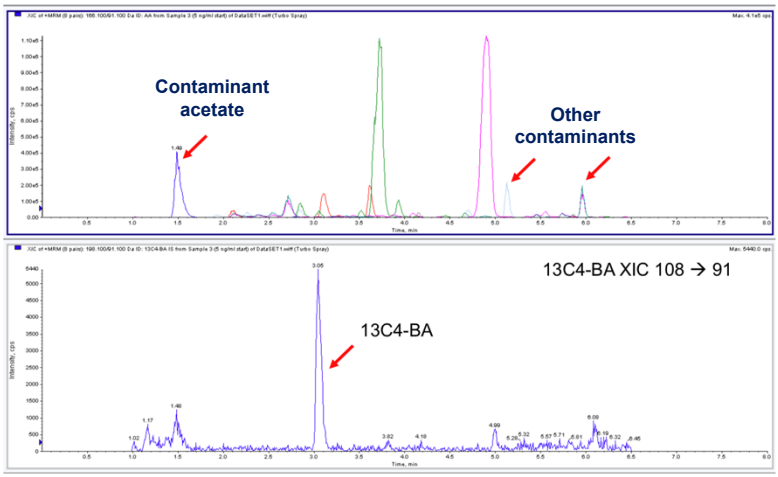


Slightly decreased serum butyrate in ERA



Contamination issues with other SCFAs

5 ng/ml starting INST concentration

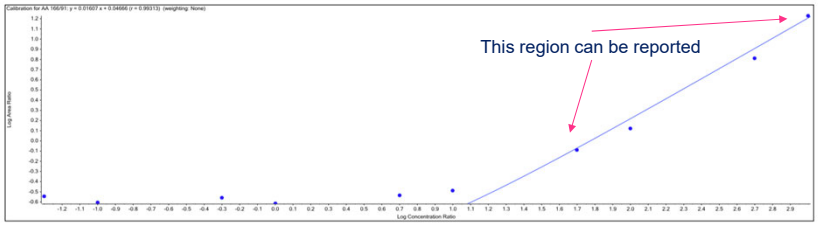


SCFA ISSUES

- During the process of trying to quantify SCFA from serum for M Stoll the following occurred:
  - Some analyte standard curves generated a signal that does not follow expected [concentration] to [signal] ratio
    - AA, PA, VA, and HA all have this issue
    - AA is the affected the most severely
  - A static amount of signal was detected in the lowest 4-5 standards in the curve
  - Since standards and samples have been contaminated their values for PA and AA are un-reliable
  - Need to determine where contamination is occurring
  - Associated with derivitization since product masses are derivative specific losses

Signal does not follow expected [concentration] to [signal] curve

Acetic Acid(AA) standard curve with contamination



Summary: TRP in ERA

- Lower diversity of fecal ions in ERA
- Decreased fecal water TRP seen x 2
- Unable to validate with targeted studies
- However, two fecal water TRP metabolites correlated with index of disease activity
- Serum TRP was lower in ERA
- Taken together, data may support role for TRP in ERA

Summary: SCFA in ERA

- Diminished abundance of butyrate-producing *F. prausnitzii* in ERA
- Diminished genetic potential to make butyrate in ERA
- Unsupervised metabolomics show decreased fecal water SCFA
- Slightly decreased serum butyric acid in ERA
  - Other SCFA are work in progress
- Taken together, data seem to support role for SCFA in ERA

Limitations

- Inability to validate TRP findings
- Low numbers of patients
- Effect of inflammatory process / therapies

Current and future directions

- Continue targeted metabolomics of SCFA
- Direct assessment of inflammatory potential of fecal water
- Consider re-submission of grant that proposed to compare TRP metabolism in ERA patients vs controls

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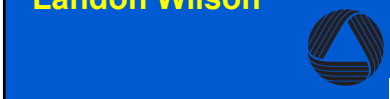

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