Ben Nichols. “Gut metabolome and microbiota signatures predict response to treatment with exclusive enteral nutrition in a prospective study in children with active Crohn’s disease”

Supplementary Table 1: Baseline demographic, anthropometric, clinical, and phenotypic characteristics of the main cohort and patient subset used in the current study[1].

Variables [1]	Main cohort (N=66)	Current study (N=37)
Sex, male n (%)	42 (64)	25 (68)
Newly Diagnosed, n (%)	60 (91)	34 (92)
Age (years), median (IQR)	13.4 (10.7, 14.9)	12.4 (10.1, 15.0)
BMI z-score, median (IQR)	-0.73 (-1.67, 0.23)	-0.70 (-1.56, 0.29)
Disease Location		
Colonic, n (%)	25 (37)	13 (35)
Ileal, n (%)	6 (9)	3 (8)
Ileocolonic, n (%)	34 (52)	21 (57)
upper GI/small bowel only, n (%)	1 (2)	0 (0)
Disease Behaviour		
B1 (inflammatory), n (%)	66 (100)	37 (100)
B2 (stricturing), n (%)	0 (0)	
B3 (penetrating), n (%)	0 (0)	
Perianal involvement, n (%)	6 (9)	4 (11)
wPCDAI, median (IQR)	42.5 (25, 60)	35.0 (22, 57.5)
FCal (µg/g), median (IQR)	1438 (1022, 1823)	1610 (1136, 1865)

[1]There were no statistically significant differences between patients enrolled in the main study and the group for which data are presented in the current study. Chi-square tests were used for categorical variables, and Mann-Whitney U tests for numerical variables. Data are presented with medians (Q1, Q3) or counts (%). GI: gastrointestinal tract, wPCDAI, weighted Paediatric Crohn’s Disease Activity Index, FCal, Faecal Calprotectin; BMI: Body mass index.

Supplementary Table 2: Pre-treatment stool characteristics, levels of targeted diet-related bacterial metabolites and microbial load, between responders and non-responders to treatment in children with Crohn's disease who completed 8 weeks of EEN.

Variables [1]	Responders N=13	Non-Responders N=16	P value
Short Chain Fatty Acids µmol/g (dry)			
Acetate	297 (250, 446)	465 (288, 548)	0.16
Propionate	72.6 (66.6, 99.1)	90.9 (53.6, 136)	0.50
Butyrate	64.9 (48.0, 86)	112 (65.7, 162)	0.04
Isobutyrate	8.04 (5.03, 12.6)	11.5 (6.95, 15.7)	0.33
Isovalerate	9.06 (5.79, 12.1)	10.4 (8.26, 14.9)	0.39
Isocaproate	0.93 (0.58, 2.16)	1.31 (0.453, 2.25)	0.84
Valerate	8.28 (1.44, 10.7)	7 (1.63, 14.8)	0.50
Caproate	0.78 (0.47, 1.68)	0.735 (0.523, 1.51)	0.91
Heptanoate	0.07 (0, 0.2)	0.085 (0.023, 0.123)	1.00
Octanoate	0.08 (0, 0.23)	0.02 (0, 0.085)	0.25
Total SCFA	482 (414, 614)	674 (489, 877)	0.10
Short Chain Fatty Acids µmol/g (wet)			
Acetate	51.6 (45.8, 79.8)	75.6 (60.4, 97.6)	0.09
Propionate	12.3 (8.5, 18.2)	16.9 (9.01, 30.1)	0.50
Butyrate	13.0 (7.79, 16.9)	20.6 (7.99, 30.5)	0.06
Isobutyrate	2.01 (0.676, 3.59)	2.29 (1.15, 3.33)	0.71
Isovalerate	2.19 (0.78, 3.25)	2.05 (1.31, 3.21)	0.75
Isocaproate	0.193 (0.154, 0.287)	0.208 (0.067, 0.444)	0.98
Valerate	1.49 (0.15, 2.68)	1.47 (0.334, 3.225)	0.62
Caproate	0.145 (0.077, 0.39)	0.137 (0.097, 0.296)	0.88
Heptanoate	0.013 (0, 0.021)	0.014 (0.003, 0.031)	0.77
Octanoate	0.013 (0, 0.077)	0.004 (0, 0.023)	0.33
Total SCFA	82.0 (72.5, 109)	133 (88.8, 165)	0.06
Hydrogen sulphide & ammonia			
Total sulphide (per wet matter),	315 (121, 686)	157 (106, 380)	0.39
Free sulphide (per wet matter),	14.4 (9.54, 25.5)	9.42 (1.21, 26.9)	0.40
NH_3 (per wet matter), mg/g	2.9 (2.05, 3.35)	3.40 (2.05, 5.00)	0.57
Faecal characteristics			
Whole stool weight	48.0 (20.9, 108)	49.5 (19.2, 75.8)	0.75
%Water content	81.3 (73.5, 86.4)	81.4 (78.0, 84.6)	0.87
Bristol Stool scale	6.0 (5.25, 7.0)	6.0 (5.0, 7.0)	0.83
Faecal pH	6.82 (6.40, 7.19)	6.66 (6.17, 7.35)	0.65
Log_{10} gene copy number/g dry	11.8 (11.7, 11.9)	11.5 (10.7, 11.8)	0.12

[1] Numerical data are presented as median (IQR); EEN: Exclusive enteral nutrition; SCFA: Short chain fatty acids. EEN: Exclusive enteral nutrition. Mann-Whitney U tests were used for statistical analysis

Supplementary Table 3: ^{1}H NMR metabolites in faeces of responders and non-responders to treatment with EEN

Metabolites (µg/g) [1]	Responders N=15	Non-Responders N=19	P value
2-Hydroxy-3-methylvalerate	1.92 (0.570, 2.65)	2.47 (1.09, 3.74)	0.238
3,4-Dihydroxyphenylacetic acid	0.013 (0.013, 0.013)	0.013 (0.013, 0.013)	NA
3-(3-Hydroxyphenyl) propionic acid	0.013 (0.013, 0.013)	0.061 (0.013, 0.308)	0.011
3-Hydroxyphenylacetate	0.013 (0.013, 0.013)	0.013 (0.013, 0.013)	NA
4-Hydroxyphenylacetate	0.013 (0.013, 0.013)	0.013 (0.013, 0.108)	0.561
Acetate	49.9 (46.3, 68.5)	70.4 (57.0, 95.5)	0.027
Alanine	7.70 (6.04, 11.1)	9.32 (6.84, 16.0)	0.256
Butyrate	13.1 (8.63, 18.4)	22.3 (12.0, 31.9)	0.030
Choline	0.12 (0.092, 0.296)	0.161 (0.11, 0.363)	0.425
Glycine	6.12 (3.41, 7.68)	5.39 (3.79, 9.64)	0.732
Isobutyrate	2.46 (0.68, 3.89)	3.85 (1.81, 4.17)	0.259
Isoleucine	3.21 (2.28, 4.55)	3.87 (2.66, 5.49)	0.410
Isovalerate	2.35 (0.83, 2.83)	3.09 (1.81, 4.01)	0.115
Leucine	4.20 (2.60, 5.47)	4.83 (3.46, 8.47)	0.157
Lysine	3.32 (2.12, 4.25)	3.38 (2.09, 6.54)	0.456
Malonate	0.714 (0.557, 0.927)	0.625 (0.53, 1.01)	0.656
Methanol	1.21 (0.722, 2.29)	1.14 (0.982, 1.40)	0.656
Phenylacetate	0.175 (0.013, 0.611)	0.943 (0.438, 1.35)	0.021
Phenylalanine	2.24 (1.67, 2.94)	2.51 (1.48, 4.04)	0.515
Propionate	16.5 (10.1, 19.8)	20.9 (15.5, 30.4)	0.051
Putrescine	0.013 (0.013, 0.882)	0.798 (0.013, 2.135)	0.192
Succinate	2.76 (0.619, 12.7)	0.84 (0.538, 2.21)	0.202
Threonine	2.37 (1.83, 2.97)	2.72 (1.54, 4.55)	0.451
Tryptophan	0.311 (0.218, 0.433)	0.353 (0.214, 0.519)	0.627
Tyrosine	1.83 (1.14, 2.00)	1.92 (1.34, 3.20)	0.202
Uracil	1.04 (0.628, 1.34)	1.22 (0.630, 2.49)	0.430
Valerate	1.91 (0.543, 3.88)	3.10 (1.25, 4.99)	0.165
Valine	4.50 (3.48, 6.47)	4.76 (3.17, 8.09)	0.732
p-Cresol	0.079 (0.020, 0.374)	0.130 (0.027, 0.245)	0.889

[1] Numerical data are presented as median (IQR); NA: non-applicable. EEN: Exclusive enteral nutrition; Mann-Whitney U tests were used for statistical analysis

Supplementary Table 4: Pre-treatment plasma cytokine concentrations of responders and non-responders to treatment in patients who completed 8 weeks of EEN

Cytokines (pg/ml) [1]	Responders N=9	Non-Responders N=21	P value
TNF-α	1.87 (1.31, 2.18)	1.54 (1.25, 1.98)	0.54
IFN-γ	20.8 (7.75, 52.9)	30.0 (5.94, 49.3)	0.71
IL-1β	0.113 (0.093, 0.187)	0.127 (0.107, 0.201)	0.41
IL-2	0.422 (0.303, 0.603)	0.312 (0.237, 0.376)	0.19
IL-4	0.026 (0.021, 0.034)	0.026 (0.019, 0.033)	0.85
IL-6	2.21 (1.53, 3.97)	2.03 (1.25, 3.84)	0.90
IL-8	6.78 (3.34, 13.0)	6.63 (4.55, 10.5)	0.65
IL-10	0.249 (0.208, 0.433)	0.269 (0.186, 0.368)	0.87
IL-12p70	0.144 (0.138, 0.162)	0.198 (0.144, 0.263)	0.16
IL-13	2.88 (2.46, 4.01)	4.01 (3.16, 6.19)	0.09
IL-17A	9.75 (6.84, 16.6)	10.6 (8.18, 14.8)	0.90
IL-17E	5.00 (2.90, 11.2)	3.98 (2.46, 5.24)	0.10
IL-17F	1305 (577, 2312)	1701 (897, 2042)	0.93
IL-21	979 (811, 1806)	1456 (982, 1654)	0.62
IL-22	2.71 (1.58, 5.61)	2.29 (1.89, 3.73)	0.62
IL-23	8.37 (4.45, 16.3)	12.1 (6.50, 13.3)	0.56
IL-27	558 (341, 833)	526 (424, 668)	0.87
IL-31	58.1 (37.4, 84.5)	69.5 (48.6, 82.9)	0.87
IL-33	3.55 (1.69, 6.49)	4.19 (2.48, 6.29)	0.87

[1] Numerical Data are presented as median (IQR). EEN: Exclusive enteral nutrition; Mann-Whitney U tests were used for statistical analysis

Supplementary Table 5: Normalised protein expression between responders and non-responders to treatment with EEN

Protein [1]	Responders N=13	Non-Responders N=18	P value
NT.3	0.69 (0.69, 0.69)	1.49 (0.69, 1.79)	0.03
CXCL6	11.1 (10.5, 11.2)	10.5 (10.3, 10.9)	0.05
TRANCE	4.07 (3.65, 4.8)	5 (4.47, 5.44)	0.06
IL.18R1	8.65 (8.32, 8.82)	8.14 (7.92, 8.52)	0.06
MMP.10	8.86 (8.56, 9.37)	9.73 (8.81, 10.4)	0.07
AXIN1	2.1 (1.93, 3.13)	2.67 (2.33, 2.93)	0.09
SIRT2	3.2 (2.99, 4.74)	3.88 (3.31, 5.08)	0.09
IL8	6.24 (5.26, 7.25)	6.76 (6.3, 7.45)	0.10
IL.15RA	1.31 (1.15, 1.55)	1.51 (1.4, 1.67)	0.11
TRAIL	7.84 (7.63, 8.11)	8.02 (7.9, 8.23)	0.11
CXCL5	11.9 (11.1, 12.6)	11.4 (10.9, 11.8)	0.11
TNFB	5.2 (5.04, 5.46)	5.52 (5.23, 5.69)	0.12
IL4	0.49 (0.49, 0.49)	0.49 (0.49, 1.47)	0.15
SLAMF1	1.01 (1.01, 1.01)	1.01 (1.01, 1.83)	0.19
MCP.3	3.15 (3, 3.74)	3.72 (3.16, 3.9)	0.20
HGF	8.34 (8.13, 8.57)	8.18 (7.91, 8.32)	0.20
LAP.TGF.beta.1	8.01 (7.51, 8.14)	7.64 (7.45, 7.92)	0.23
NRTN	0.66 (0.66, 0.66)	0.66 (0.66, 0.66)	0.24
CD5	5.74 (5.34, 5.89)	6.02 (5.49, 6.11)	0.24
SCF	8.99 (8.32, 9.43)	8.61 (8.13, 8.96)	0.26
CASP.8	2.45 (2.19, 2.83)	2.66 (2.17, 3.12)	0.26
FGF.5	0.66 (0.66, 0.66)	0.66 (0.66, 0.66)	0.27
Beta.NGF	0.69 (0.69, 0.69)	0.69 (0.69, 0.69)	0.27
IL33	0.89 (0.89, 0.89)	0.89 (0.89, 0.89)	0.27
LIF	0.54 (0.54, 0.54)	0.54 (0.54, 0.54)	0.27
MCP.1	11.7 (11.3, 11.8)	11.8 (11.5, 11.9)	0.31
CD40	11.2 (11.0, 11.5)	11.3 (11.2, 11.6)	0.31
STAMBP	4.12 (3.98, 5.1)	4.49 (4.11, 5.67)	0.31
CCL28	0.86 (0.39, 1.09)	1.03 (0.87, 1.27)	0.32
IL.10RB	6.12 (5.97, 6.3)	6.3 (5.95, 6.45)	0.33
EN.RAGE	3.62 (3.25, 4.5)	4.23 (3.56, 4.6)	0.33
GDNF	0.97 (0.97, 0.97)	0.97 (0.97, 0.97)	0.35
IL.22.RA1	1.1 (1.1, 1.1)	1.1 (1.1, 1.1)	0.36
DNER	9.12 (8.7, 9.43)	8.82 (8.57, 9.04)	0.42
IL.20RA	0.73 (0.73, 0.73)	0.73 (0.73, 0.73)	0.43
IL.17A	2.61 (2.36, 2.86)	2.89 (2.19, 3.53)	0.44
IL6	4.41 (3.84, 5.17)	4.17 (3.52, 4.47)	0.46
CST5	5.19 (5, 5.41)	5.01 (4.85, 5.28)	0.49
Flt3L	8.25 (7.85, 8.46)	8.19 (7.82, 8.29)	0.49
X4E.BP1	7.31 (6.86, 8.43)	8.03 (6.98, 9.24)	0.49
CXCL1	9.53 (8.8, 9.93)	9.4 (9.04, 9.69)	0.54
MCP.2	8.31 (7.89, 8.87)	8.68 (8.14, 8.87)	0.54
IL.10RA	0.51 (0.51, 1.24)	1.06 (0.51, 1.35)	0.59
CSF.1	10.1 (10.0, 10.3)	10.0 (9.97, 10.3)	0.59

MMP.1	9.57 (8.75, 10.6)	9.29 (8.92, 9.92)	0.62
IL.24	1.09 (1.09, 2.44)	1.09 (1.09, 2.63)	0.64
IL7	2.79 (2.64, 3.11)	2.7 (2.63, 3.04)	0.65
LIF.R	4.06 (3.83, 4.27)	4.06 (3.93, 4.25)	0.65
CCL20	7.68 (6.52, 8.11)	7.57 (7.33, 8.07)	0.65
IL5	0.79 (0.79, 0.79)	0.79 (0.79, 0.79)	0.66
FGF.21	4.68 (3.91, 5.39)	4.72 (3.88, 6.76)	0.69
OPG	9.36 (9.29, 9.62)	9.51 (9.31, 9.63)	0.74
uPA	10.1 (9.84, 10.4)	10.2 (9.92, 10.3)	0.74
TNFSF14	4.33 (3.62, 4.54)	3.95 (3.65, 4.68)	0.74
CCL19	9.1 (8.99, 9.99)	9.37 (9.06, 9.86)	0.74
PD.L1	6.65 (6.45, 6.96)	6.6 (6.43, 6.95)	0.74
IL.17C	0.9 (0.9, 1.81)	0.9 (0.9, 1.99)	0.74
ARTN	0.36 (0.36, 0.36)	0.36 (0.36, 0.36)	0.75
CD8A	10.5 (10.3, 11.1)	10.6 (10.3, 11.0)	0.77
CXCL11	13.0 (12.3, 13.5)	13.1 (12.7, 13.3)	0.77
TGF.alpha	3.79 (3.52, 4.01)	3.79 (3.56, 3.99)	0.77
FGF.23	2.55 (2.36, 2.93)	2.75 (2.44, 2.95)	0.77
IL.12B	6.67 (6.36, 6.98)	6.71 (6.43, 7.01)	0.77
TNF	3.93 (3.52, 4.27)	3.97 (3.7, 4.2)	0.77
CD244	7.17 (6.97, 7.34)	7.16 (6.98, 7.43)	0.80
MCP.4	14.9 (14.7, 15.1)	14.8 (14.5, 15.4)	0.80
CCL11	7.48 (7.26, 7.72)	7.41 (7.3, 7.77)	0.80
IL10	3.85 (3.53, 4.83)	3.91 (3.72, 4.32)	0.80
IFN.gamma	8.57 (7.4, 10.11)	8.9 (7, 9.93)	0.80
FGF.19	7.01 (6.87, 7.26)	7.26 (6.7, 7.71)	0.80
ST1A1	3.39 (3, 3.55)	3.37 (2.96, 3.79)	0.80
CCL4	6.46 (6.11, 6.66)	6.27 (6.14, 6.62)	0.83
CCL3	5.41 (4.73, 5.68)	5.19 (4.93, 5.51)	0.83
TNFRSF9	8.3 (8.04, 8.45)	8.3 (7.95, 8.55)	0.83
ADA	5.14 (4.99, 5.89)	5.55 (5.14, 5.86)	0.83
CCL25	5.86 (5.41, 6.56)	6 (5.63, 6.27)	0.86
CX3CL1	5.63 (5.55, 5.79)	5.64 (5.45, 5.81)	0.86
IL.2RB	0.85 (0.85, 0.85)	0.85 (0.85, 0.85)	0.86
TSLP	0.31 (0.31, 0.31)	0.31 (0.31, 0.31)	0.89
CDCP1	2.98 (2.7, 3.59)	2.97 (2.83, 3.26)	0.89
CD6	5.36 (5.32, 5.73)	5.35 (4.96, 5.99)	0.89
VEGFA	9.95 (9.72, 10.4)	10.1 (9.71, 10.3)	0.95
CCL23	10.7 (10.3, 11.1)	10.8 (10.3, 11.1)	0.95
OSM	4.04 (3.57, 4.63)	4.26 (3.25, 4.84)	0.98
CXCL9	9.6 (8.45, 10.6)	9.72 (8.86, 10.2)	0.98
IL18	8.98 (8, 9.43)	8.98 (8.56, 9.37)	0.98
CXCL10	10.3 (8.97, 10.5)	10.0 (9.34, 10.5)	1.00
TWEAK	9.56 (9.29, 9.62)	9.48 (9.27, 9.65)	1.00
IL.1.alpha	0.18 (0.18, 0.18)	0.18 (0.18, 0.18)	NA
IL2	0.86 (0.86, 0.86)	0.86 (0.86, 0.86)	NA
IL13	0.81 (0.81, 0.81)	0.81 (0.81, 0.81)	NA
IL.20	0.55 (0.55, 0.55)	0.55 (0.55, 0.55)	NA

[1] Numerical Data are presented as median (IQR); NA: non-applicable; EEN: exclusive enteral nutrition; Mann-Whitney U tests were used for statistical analysis