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Microbiota signature differs significantly between NAFLD and healthy Controls but not between NAFL and NASH

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Background and Aims: Previous studies have shown that gut dysbiosis is associated with non-alcoholic fatty liver disease (NAFLD). However, the data regarding differences in microbiota composition between the two NAFLD phenotypes, i.e. non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) are inconsistent. In this prospective cross-sectional study we aimed to evaluate the association between gut bacterial dysbiosis and the presence of NAFLD in general and NAFL and NASH in particular.

Method: Ninety patients with NAFLD (n=21 NAFL, n=47 NASH; n=23 without liver biopsy) and 21 healthy controls (HC) were enrolled. Taxonomic composition of gut microbiota was determined using 16S rDNA gene sequencing of stool samples. Linear discriminant analysis (LDA) effect size (LEfSe) analysis, followed by multivariate logistic regression was performed to detect differences in the bacterial composition of gut microbiota between the groups.

Results: Mean age of all study subjects (n=111) was 44.5 years (SD ± 14.3) and 54 (49 %) were women. Comparing NAFLD to HC we observed three phyla and nine families to differ significantly (p<0.05) between the two groups. After multivariate analysis only the differences in abundance of *Bacteroidetes* (phylum) and *Ruminococcaceae* (family) between NAFLD and HC remained significant. LEfSe found no phyla but twelve families to be significantly different between biopsy-proven NAFL and NASH (p<0.05). However, after multivariate logistic regression (adjusted for the presence of metabolic syndrome) the difference in gut microbiota composition between NAFL and NASH was no longer statistically significant.

Conclusion: The gut microbiota composition seems to differ significantly between NAFLD and HC but not between NAFL and NASH. Thus, whereas bacterial gut microbiota signature could serve as a biomarker for the diagnosis of NAFLD, this might not be sufficient to accurately distinguish between NAFL and NASH.

Characteristics of the study population

	Healthy Controls (n=21)	NAFLD (n=90)	p-value	NAFL (n=21)	NASH (n=47)	p-value
Mean age ± SD [years]	41.4 ± 14.1	47.6 ± 14.5	0.08	43.4 ± 15.6	52.1 ± 14	0.01
Female gender, n (%)	16 (76.2)	38 (42.2)	0.01	7 (35)	22 (46.8)	0.02
Albumin [35-52 g/L]	45 (43-46)	45 (34-51)	0.87	46 (40-51)	44 (34-51)	0.11
Aspartate aminotransferase (AST) [<50 U/L]	24 (14-27)	33.5 (13-143)	0.00	30.5 (21-75)	38 (17-143)	0.00
Alanine aminotransferase (ALT) [<50 U/L]	13 (9-27)	50.5 (14-232)	< 0.0001	42 (20-177)	55 (16-232)	< 0.0001
Gamma-glutamyl-transferase (GGT) [<60 U/L]	12.5 (8-29)	80 (13-660)	< 0.0001	82.5 (14-660)	81 (17-401)	< 0.0001
Alkaline Phosphatase (AP) [40-130 U/L]	61 (47-83)	72 (42-160)	0.04	75 (43-146)	74 (43-160)	0.07
Total bilirubin [< 1.2 mg/dL]	0.5 (0.2-1.8)	0.5 (0.1-2.6)	0.49	0.6 (0.3-2)	0.5 (0.2-2.1)	0.44
Ferritin [30-400 µg/L]	81 (14-124)	203.5 (16-1260)	0.00	144.5 (19-592)	236 (16-816)	0.00
Platelet counts [150-400 x109/L]	225 (189-283)	222.5 (60-386)	0.95	197.5 (132-386)	223 (60-373)	0.87
Prothrombin time (Quick) [70-120 %]	109 (101-125)	108.5 (26-125)	0.71	103.5 (86-120)	108 (26-125)	0.74
Prothrombin time (INR) [2.0-4.5]	1 (0.8-1)	1 (0.9-2)	0.79	1 (0.9-1.1)	1 (0.9-2)	0.75
Fasting glucose[74-109 mg/dL]	75.5 (46-84)	96.5 (63-200)	< 0.0001	93.5 (76-147)	102 (63-200)	< 0.0001
HbA1c [28-38 mmol/mol]	30 (29-33)	35.8 (27-68)	0.04	33 (27-41)	37.5 (27-64)	0.00
Insulin [2.6-24.9 mU/L]	33.4 (33.4-33.4)	21.7 (3-274.2)	n/a	9.2 (5.6-68.7)	33.4 (3-274.2)	n/a
Triglycerides [>200 mg/dL]	106 (59-255)	139.5 (29-550)	0.30	106.5 (29-253)	150 (55-484)	0.09
Total cholesterol [<200 mg/dL]	169.5 (135-229)	186 (86-329)	0.24	177 (86-272)	183 (96-329)	0.38
High density lipoprotein (HDL) [35-55 mg/dL]	68 (60-106)	48 (27-88)	0.03	53 (33-82)	44 (27-88)	0.01
Low density lipoprotein (LDL) [<150 mg/dL]	65 (51-95)	119 (15-247)	0.05	105.5 (15-184)	113 (24-247)	0.18
25-OH Vitamin D [30-70 µg/L]	21.1 (15.4-44.4)	18.2 (4.5-49.9)	0.16	16.3 (4.9-37.4)	18 (4.5-49.4)	0.23
Body mass index [kg/m²]	20 (19.2-22.9)	29.9 (20.5-46.5)	< 0.0001	28.5 (22.5-35)	31.2 (24.6-46.5)	< 0.0001
Waist circumference [cm]	85 (78-87)	106 (81-143)	< 0.0001	100 (84-128)	109 (81-143)	< 0.0001
Dyslipidemia, n (%) [yes]	2 (25)	51 (56.7)	0.18	8 (40)	30 (63.8)	0.05
Type 2 diabetes, n (%) [yes]	0 (0)	18 (20)	0.36	0 (0)	14 (29.8)	0.01
Arterial hypertension, n (%) [yes]	0 (0)	49 (54.4)	0.01	6 (30)	32 (68.1)	0.00
Systolic blood pressure [mmHg]	119 (100-130)	135 (112-183)	0.00	130 (118-165)	136 (112-183)	0.00
Diastolic blood pressure [mmHg]	70 (59-85)	83.5 (63-114)	0.00	80.5 (66-102)	86 (63-114)	0.00
Metabolic syndrome, n (%) [yes]	0 (0)	30 (33.3)	0.12	2 (10)	21 (44.7)	0.00
Alcohol consumption [g/d]	2 (2-14)	1 (0-30)	0.66	3 (0-12)	1 (0-30)	0.06
NAFLD activity score (NAS)	n/a	4 (1-8)	n/a	2.5 (1-3)	5 (3-8)	n/a
Fibrosis stage (liver biopsy)	n/a	1 (0-4)	n/a	0 (0-1)	2 (0-4)	n/a
Fibrosis stage (liver biopsy) 0, n (%)				12 (60)	4 (8.5)	0.00
Fibrosis stage (liver biopsy) 1, n (%)				8 (40)	14 (29.8)	0.00
Fibrosis stage (liver biopsy) 2, n (%)				0 (0)	11 (23.4)	0.00
Fibrosis stage (liver biopsy) 3, n (%)				0 (0)	4 (8.5)	0.00
Fibrosis stage (liver biopsy) 4, n (%)				0 (0)	14 (29.8)	0.00
Transient Elastography [kPa]	4.6 (3.4-5.5)	5.4 (2.8-66.4)	0.00	4.5 (2.8-7.6)	7.6 (2.9-66.4)	< 0.0001
AST to Platelet Ratio Index (APRI)	0.2 (0.1-0.3)	0.3 (0.1-2.1)	< 0.0001	0.3 (0.1-0.7)	0.4 (0.1-2.1)	0.00
FIB4	1 (0.5-1.8)	1 (0.3-8.1)	0.66	1 (0.4-2.7)	1.2 (0.3-8.1)	0.10
NAFLD fibrosis score (NFS)	-2.7 (-4.4- -1.6)	-2.3 (-5.1-2.7)	0.13	-3.1 (-4.4- -0.2)	-1.6 (-5.1-2.7)	0.01
PNPLA3 p.I148M genotypes, CC, n (%)	6 (75)	38 (44.2)	0.21	11 (55)	15 (34.1)	0.18
PNPLA3 p.I148M genotypes, CG, n (%)	2 (25)	35 (40.7)	0.21	6 (30)	20 (45.5)	0.18
PNPLA3 p.I148M genotypes, GG, n (%)	0 (0)	13 (15.1)	0.21	3 (15)	9 (20.5)	0.18
Chao1	108 (82-176)	112.7 (67-176.4)	0.57	110.6 (73.1-156)	112.1 (67-159.1)	0.94
Inverse Simpson	18.9 (12.2-31.6)	17.5 (2.6-33.4)	0.20	20 (13.5-25.3)	16.9 (4.5-33.4)	0.13
Observed OTUs	105 (79-155)	108 (66-153)	0.62	108.5 (71-131)	107 (67-141)	0.95
Pielou's evenness (PE)	0.7 (0.7-0.8)	0.7 (0.5-0.8)	0.15	0.8 (0.7-0.8)	0.7 (0.5-0.8)	0.21
Shannon Index	3.5 (3.2-3.9)	3.4 (2.1-3.9)	0.40	3.5 (3-3.8)	3.3 (2.3-3.9)	0.12

Table 1: Characteristics of the study population. Data refers to the baseline visit. Reference values of laboratory parameters are given in squared brackets []. Values are given as median with range in round brackets () if not stated otherwise.

Linear discriminant analysis (LDA) effect size (LEfSe) analysis

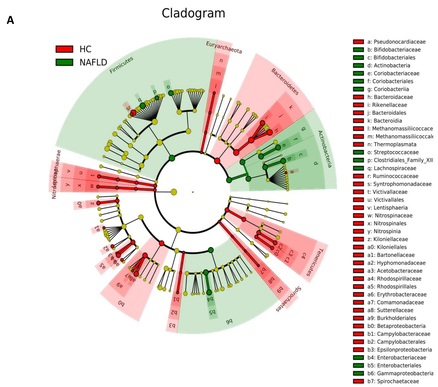

Figure 1: Differently abundant taxa between NAFLD and Healthy Controls (A) and NAFL and NASH (B) identified by linear discriminant analysis effect size (LEfSe) analysis. Named taxa indicate an increased abundance in the corresponding group. **A:** Healthy controls -> red; NAFLD -> green; **B:** NAFL -> red, NASH -> green.

Multivariate logistic regression

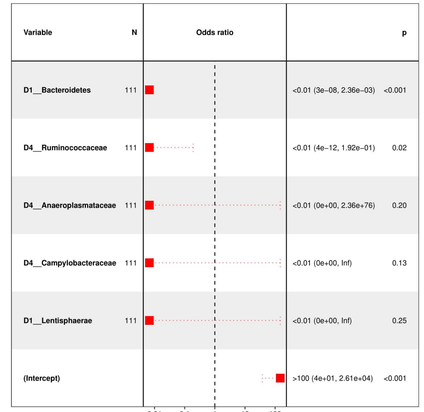

Table 2: Significantly different abundant taxa after multivariate logistic regression comparing NAFLD and Healthy Controls. Adjusted for metabolic syndrom.

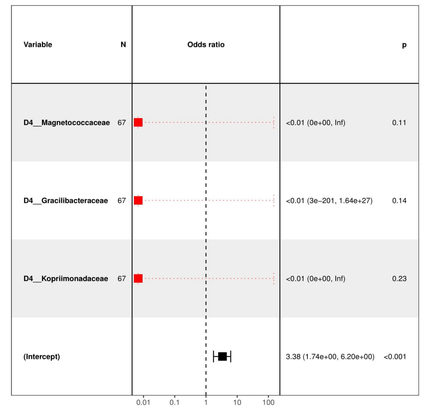

Table 3: No significantly different abundance of taxa after multivariate logistic regression comparing NAFL and NASH. Adjusted for metabolic syndrom.

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