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**Mining possible associations of faecal *A. muciniphila* colonisation patterns with host adiposity and cardiometabolic markers in an adult population**

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**Table S1. Primers and qPCR characteristics of gut microbiota analysis.[1]**

| Target | Primer | Primer sequence (5'-3') | Annealing temperature (°C) | Product Size (bp) | Reference strains | References |
|---|---|---|---|---|---|---|
| Total Bacteria (Universal) | Forward<br>Reverse | TCCTACGGGAGGCAGCAGT<br>GGACTACCAGGGTATCTAATCCTGTT | 60°C | 466bp | *Bacteroides fragilis* MM44 (ATCC 25285) | Nadkarni *et al*., 2002 |
| *Akkermansia muciniphila* | AM1<br>AM2 | CAGCACGTGAAGGTGGGGAC<br>CCTTGCGGTTGGCTTCAGAT | 60°C | 327bp | *Akkermansia muciniphila* DSM 22959 | Collado *et al*., 2007 Collado *et al*., 2008 |
| Firmicutes | Firm934F<br>Firm1060R | GGAGYATGTGGTTTAATTCGAAGCA<br>AGCTGACGACAACCATGCAC | 60°C | 126bp | *Clostridium perfringens* ATCC 13124 | Guo *et al*., 2008 |
| Bacteroidetes | Bact934F<br>Bact1060R | GGARCATGTGGTTTAATTCGATGAT<br>AGCTGACGACAACCATGCAG | 60°C | 126bp | *Bacteroides fragilis* MM44 (ATCC 25285) | Guo *et al*., 2008 |
| *Bacteroides* spp. | Bac303F<br>Bfr-Fmrev | GAAGGTCCCCCACATTG<br>CGCKACTTGGCTGGTTCAG | 60°C | 103bp | *Bacteroides fragilis* MM44 (ATCC 25285) | Ramirez-Farias *et al*., 2009 |
| *Prevotella* spp. | g-Prevo-F<br>g-Prevo-R | CACRGTAAACGATGGATGCC<br>GGTCGGGTTGCAGACC | 55°C | 513bp | *Prevotella copri* DSM 18205 | Matsuki *et al*., 2002 |
| *Clostridium coccoides* group | F_Ccoc07<br>R_Ccoc14 | GACGCCGCGTGAAGGA<br>AGCCCCAGCCTTTCACATC | 55°C | 199bp | *Blautia producta* DSM 2950 | Furet *et al*., 2009 Furet *et al*., 2010 |
| *Clostridial* cluster IV (*Clostridium leptum* group) | Clep866mF<br>Clept1240mR | TTAACACAATAAGTWATCCACCTGG<br>ACCTTCCTCCGTTTTGTCAAC | 55°C | 314bp | *Faecalibacterium prausnitzii* DSM 17677 | Ramirez-Farias *et al*., 2009 |
| *Clostridium perfingens* group | CPF<br>CPR | ATGCAAGTCGAGCGATG<br>TATGCGGTATTAATCTCCCTTT | 55°C | 120bp | *Clostridium perfringens* ATCC 13124 | Phong *et al*., 2010 |

| Target | Primer | Primer sequence (5'-3') | Annealing temperature (°C) | Product Size (bp) | Reference strains | References |
|---|---|---|---|---|---|---|
| *Lactobacillus* group | Forward | AGCAGTAGGGAATCTTCCA | 58°C | 341bp | *Lactobacillus gasseri* | Rinttilä *et al*, |
| | Reverse | CACCGCTACACATGGAG | | | DSM 20243 | 2004 |
| *Bifidobacterium* spp. | Forward | TCGCGTCYGGTGTGAAAG | 58°C | 243bp | *Bifidobacterium bifidum* | Rinttilä *et al*., |
| | Reverse | CCACATCCAGCRTCCAC | | | DSM 20456 | 2004 |
| *Escherichia coli* subgroup | Forward | GTTAATACCTTTGCTCATTGA | 61°C | 340bp | *Escherichia coli* | Malinen *et al.*, |
| | Reverse | ACCAGGGTATCTAATCCTGTT | | | ATCC 25922 | 2003 |

[1] Real-time quantitative PCR (qPCR) amplification and detection were performed in a LightCycler® 2.0 Real-Time PCR System (Roche Diagnostics GmbH, Germany). All PCR tests were carried out in duplicate with a final volume of 20 µl per reaction, containing 10 ng of each faecal DNA preparation (2 ng/µl), 10µl of KAPA SYBR® Fast Master Mix (2×) Universal Kit (Kapa Biosystems Inc., Wilmington, USA), 200 nM of each primer, 0.25 µl of Bovine Serum Albumin (BSA 20mg/ml, New England Biolabs Inc, UK) for minimization of reagent abstraction on glass capillaries surface and 3.95 µl PCR-grade water. The thermal cycling conditions included an initial enzyme activation step at 95 ºC for 3 min, followed by 45 cycles of DNA denaturation at 95 °C for 3 s, primer annealing at optimal annealing temperature for 20 s and extension at 72 ºC for the minimum time required for data acquisition at 72 ºC according to instrument guidelines [template size (bp)/25]. Melting curve analysis was performed by slowly cooling the PCRs from 95 ºC to 65ºC (0.1 ºC/s) with simultaneous measurement of the SYBR Green I signal intensity. Melting-point determination analysis allowed the confirmation of the specificity of the amplification products. Microbial quantification was based on standard curves of genomic DNA from reference strains performed with the LightCycler® software version 4.1 (Roche Diagnostics GmbH).

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**Table S2. Subjects' gut microbiota analysis according to bimodal levels of *Akkermansia muciniphila*.[1,2]**

| **Microorganisms** | ***A. muciniphila* bimodal levels** | | |
|---|---|---|---|
| | **Low levels (n=55)** | **High levels (n=54)** | ***P*-value** |
| *Firmicutes* | 11.67±0.21 | 11.73±0.21 | 0.124 |
| *Bacteroidetes* | 11.08±0.27 | 11.12±0.25 | 0.443 |
| *Firmicutes*-to-*Bacteroidetes* ratio | 1.06 (1.04-1.07) | 1.06 (1.04-1.07) | 0.884 |
| *Bacteroides* spp. | 11.02±0.31 | 11.05±0.28 | 0.626 |
| *Prevotella* spp. | 8.85 (8.57-10.67) | 9.02 (8.72-10.47) | 0.463 |
| *Clostridium coccoides group* | 10.75±0.21 | 10.81±0.26 | 0.174 |
| *Clostridial cluster IV (Clostridium leptum group)* | 10.72±0.27 | 10.80±0.26 | 0.121 |
| *Clostridium perfringens* group | 8.08±0.74 | 8.25±0.69 | 0.205 |
| *Lactobacillus* group | 7.81 (7.32-8.51) | 7.79 (7.41-8.37) | 0.827 |
| *Bifidobacterium* spp. | 10.52 (10.01-10.84) | 10.57 (10.23-10.87) | 0.370 |
| *Escherichia coli* subgroup | 7.88±1.28 | 8.05±1.12 | 0.485 |

[1] Subjects were assigned to low (<8.79 $\log_{10}$ copies of 16S/g wet faeces) or high (≥8.79$\log_{10}$ copies of 16S/g wet faeces) bimodal levels of *A. muciniphila*, based on median value.

[2] Values are $\log_{10}$ copies of 16S rRNA gene /g wet faeces, expressed as mean and standard deviation (SD) for parametric or median and interquartile range ($25^{th}$-$75^{th}$ percentile) for nonparametric data; Values within a row were significantly different if *P*-value <0.05.

**Table S3. Multiple linear regression analysis among faecal *Akkermansia muciniphila* characteristics and indexes of metabolic health.[1,2,3]**

| ***Biochemical profile*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Model for** | **TC ($log_{10}$)** | **HDL-C** | **LDL-C ($log_{10}$)** | **TG ($log_{10}$)** | **TC/HDL-C ratio ($log_{10}$)** | **LDL-C/HDL-C ratio** | **FBG ($log_{10}$)** | **Insulin ($log_{10}$)** | **HOMA-IR ($log_{10}$)** |
| *A. muciniphila* (per 1-$log_{10}$ unit) | -0.006±0.005, *P*=0.177 | 0.413±0.604, *P*=0.496 | -0.009±0.006, *P*=0.131 | -0.018±0.011, *P*=0.092 | -0.009±0.004, *P*=0.033 | -0.053±0.033, *P*=0.108 | -0.004±0.003, *P*=0.118 | -0.015±0.012, *P*=0.222 | -0.019±0.013, *P*=0.153 |
| Low vs. High *A. muciniphila* levels (presence of low levels) | 0.024±0.016, *P*=0.154 | -0.450±2.148, *P*=0.835 | 0.032±0.021, *P*=0.145 | 0.057±0.037, *P*=0.131 | 0.029±0.016, *P*=0.072 | 0.157±0.117, *P*=0.183 | 0.018±0.009, *P*=0.037 | 0.068±0.042, *P*=0.114 | 0.084±0.047, *P*=0.074 |
| Detection of *A. muciniphila* (positive) | -0.017±0.024, *P*=0.490 | 4.162±3.135, *P*=0.187 | -0.046±0.031, *P*=0.144 | 0.002±0.053, *P*=0.967 | -0.049±0.023, *P*=0.036 | -0.407±0.176, *P*=0.023 | 0.002±0.013, *P*=0.870 | 0.068±0.060, *P*=0.259 | 0.067±0.066, *P*=0.309 |
| ***Inflammation indexes*** | | | | | | | | | |
| **Model for** | **WBC ($log_{10}$)** | **Serum uric acid** | **Serum CRP ($log_{10}$)** | **Serum IL-6 ($log_{10}$)** | **Serum IL-10 ($log_{10}$)** | **Serum total adiponectin ($log_{10}$)** | **Plasma LBP** | **Plasma sCD14 ($log_{10}$)** | **Plasma LBP/sCD14 ratio** |
| *A. muciniphila* (per 1-$log_{10}$ unit) | -0.010±0.006, *P*=0.065 | 0.023±0.081, *P*=0.775 | 0.024±0.024, *P*=0.328 | -0.005±0.017, *P*=0.775 | -0.009±0.024, *P*=0.709 | 0.046±0.015, *P*=0.002 | 0.115±0.199, *P*=0.566 | 0.012±0.004, *P*=0.003 | -0.191±0.136, *P*=0.163 |
| Low vs. High *A. muciniphila* levels (presence of low levels) | 0.021±0.019, *P*=0.273 | -0.204±0.288, *P*=0.482 | 0.014±0.088, *P*=0.871 | 0.060±0.062, *P*=0.334 | 0.077±0.083, *P*=0.356 | -0.131±0.053, *P*=0.016 | 0.589±0.712, *P*=0.827 | -0.026±0.014, *P*=0.070 | 0.927±0.481, *P*=0.057 |
| Detection of *A. muciniphila* (positive) | 0.013±0.029, *P*=0.660 | -0.287±0.412, *P*=0.487 | -0.037±0.127, *P*=0.772 | 0.015±0.093, *P*=0.874 | 0.325±0.131, *P*=0.015 | -0.011±0.077, *P*=0.882 | 0.031±0.997, *P*=0.975 | -0.031±0.020, *P*=0.136 | 0.583±0.682, *P*=0.394 |

| ***Oxidative stress and other cardiometabolic markers***<br>**Model for** | **Serum TBARs ($log_{10}$)** | **Serum GPx activity ($log_{10}$)** | **Serum Lp-$PLA_2$ activity** |
|---|---|---|---|
| *A. muciniphila*<br>(per 1-$log_{10}$ unit) | -0.010±0.009, *P*=0.265 | 0.008±0.010, *P*=0.399 | -0.530±0.388, *P*=0.175 |
| Low vs. High *A. muciniphila* levels (presence of low levels) | 0.027±0.033, *P*=0.406 | -0.005±0.034, *P*=0.890 | 1.590±1.394, *P*=0.257 |
| Detection of *A. muciniphila* (positive) | 0.008±0.047 *P*=0.871 | -0.007±0.052, *P*=0.890 | 0.431±2.080, *P*=0.836 |

[1] All models were adjusted for sex, age, BMI, diagnosis/drug treatment of disease (dyslipidemia/diabetes/hypertension), current smoking, energy intake, MedDietScore, total physical activity and stool consistency (BSS).
[2] Values are expressed as beta-coefficients ± Standard Error (SE) and *P*-values.
[3] Non-parametric dependent parameters were $log_{10}$-transformed before linear regression analysis.
[4] FBG: Fasting Blood Glucose; GPx: Glutathione peroxidase; HDL-C: High-Density Lipoprotein Cholesterol; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; CRP: C-reactive protein; LBP: Lipopolysaccharide Binding Protein; LDL-C: Low-Density Lipoprotein Cholesterol; Lp-$PLA_2$:Lipoprotein-associated phospholipase $A_2$; IL-6: Interleukin-6; IL-10: Interleukin-10; sCD14: soluble Cluster of Differentiation-14; TBARS: Thiobarbituric Acid Reactive Substances; TC: Total Cholesterol; TG: Triglycerides; WBC: White Blood Cells.