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The oral metabolome and microbiome as risk factors for developing type 2 diabetes

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Aim:

Investigate the association between the composition of the oral microbiota and individuals at either high or low risk of developing diabetes and other phenotypes such as smoking. Furthermore, assess the interplay between the microbiome and metabolome.

Introduction:

Type 2 diabetes (T2D) is a serious concern to global public health. The gut microbiota may play a role in the pathogenesis of type 2 diabetes (T2D). However, little is known about the possible pathways through which the oral microbiome and metabolome may influence risk of developing T2D.

Methods:

ADDITION-PRO is a longitudinal cohort study, which consist of individuals at various risk of developing diabetes. Microbiome and metabolome profiles were generated from stimulated saliva samples, on a subset of 654 participants, using 16S rRNA gene sequencing and liquid chromatography-mass spectrometry, respectively. The sequencing results were processed by DADA2 generating an amplicon sequence variant (ASV) table annotated to the Human Oral Microbiome Database (eHOMD) obtaining 164 genera. Metabolite identification were performed with the NIST, MoNA and METLIN databases obtaining 224 metabolites. The participants were stratified into being at low risk (individuals with normal glucose tolerance; n=365) and high risk (individuals with fasting plasma glucose 6.1-6.9 mmol/L, two hour fasting blood glucose 7.8-11.0 mmol/L and/or hemoglobin A_{1c} 42-47 mmol/L ; n=271).

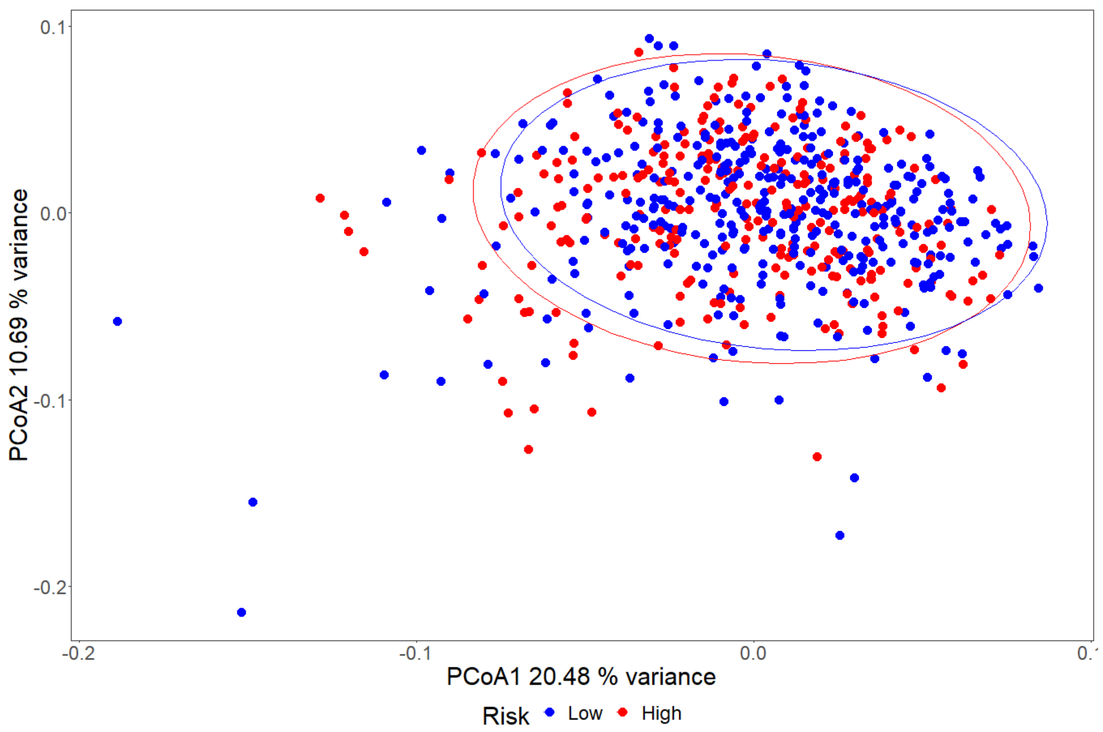

Fig 1: Plot of the principal coordinates analysis (PCoA). Points and elipses were colored according to being at high or low risk of developing diabetes. Elipses are drawn based on a 95% confidence level.

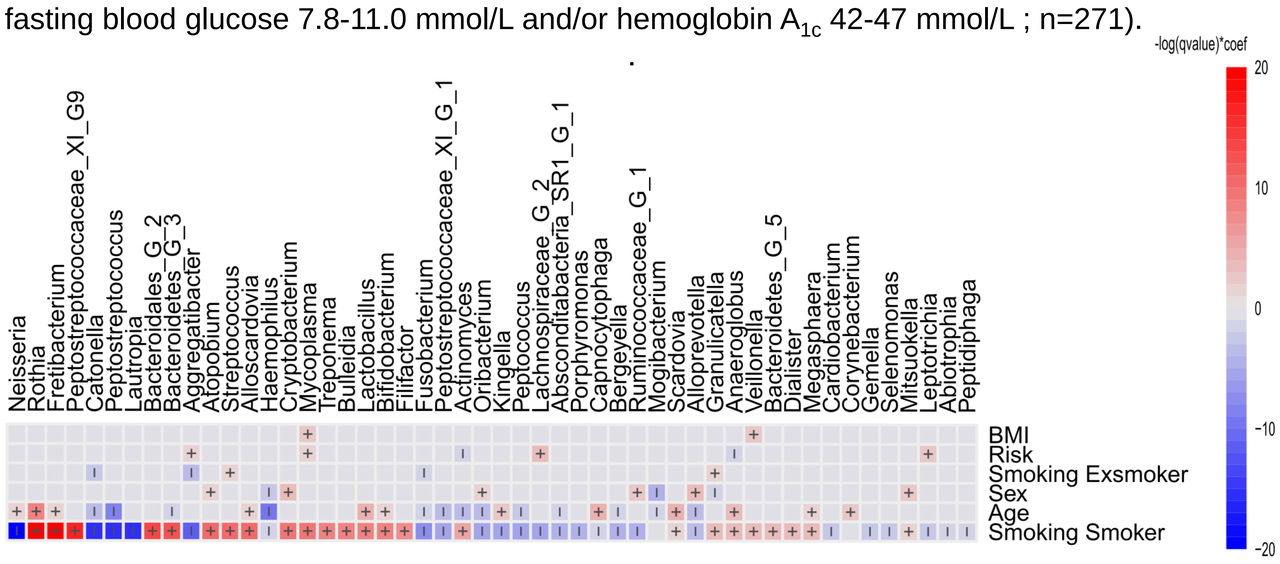

Fig 2: Heatmap of the top 50 significant microbiome (genera as outcome) associations. Multivariate analysis with linear models were performed with (MaAsLin2), including variables: Risk group, smoking status, age, sex, and BMI.

Results:

Principal Coordinates Analysis (PCoA) showed no significant differences in oral microbial composition between risk groups (PERMANOVA p=0.15) (Fig 1). In a Multivarite Analysis with Linear Models (MaAsLin) the genera *Aggregatibacter,* (Wald p=0.03), *Leptotrichia* (Wald p=0.02) and *Lachnospiraceae_G_2* (Wald p=0.004) were significantly lower in the high risk group, while the abundance of *Anaeroglobus* (Wald p=0.04) was significantly higher in the high risk group (Fig 2). Correlation analysis of the aforementioned genera, included *Leptotrichia* with L-phenylalanine (Spearman's ρ = 0.29, p=4.0*10^{-14}) and *Lachnospiraceae_G_2* with hippuric acid (Spearman's ρ = 0.30, p=8.5*10^{-15}) (Fig 3). However, these two metabolites were not different in the two groups at various risk of developing diabetes.

The variable smoking revealed a highly significant difference in microbial composition (PERMANOVA p>0.001), and also associated with a large number of genera (Fig 2).

Key points:

- Smoking showed a significant effect on the composition of the oral microbiota, but risk of developing T2D did not.
- A few significant associations between certain genera of oral bacteria with being at either high or low risk of devveloping T2D were found, indicating that certain genera may influence risk of developing T2D.
- Two of these genera were further associated with metabolites, which may help elucidate their effect on the risk of T2D.
- More studies are needed to confirm these findings and further explore the mechanisms through which the oral microbiota may affect the risk of developing T2D. The ADDITION-PRO cohort is relevant for this purpose since the cohort can be enriched with follow-up data. Furthermore, targeting an untreated group eliminates possible confounders.

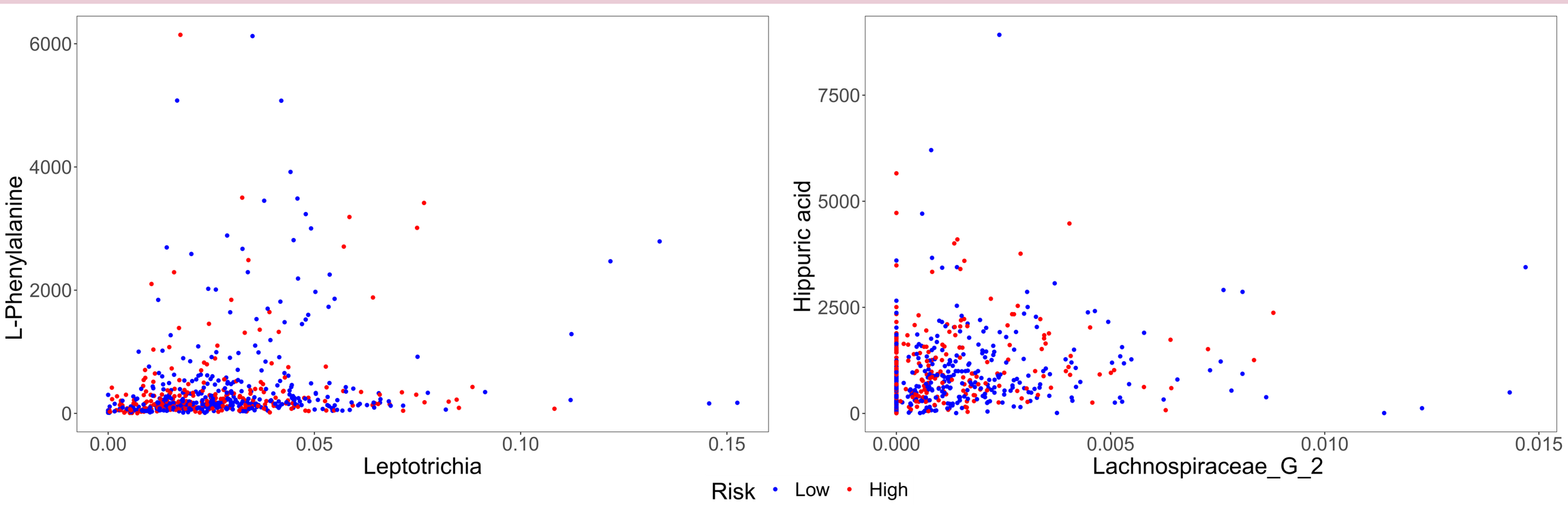

Fig 3: XY-scatter plot displaying the association between *Leptotrichia* and L-Phenylalanine, and between *Lachnospiraceae_G_2* and Hippuric acid .