Abstract # 260

Functional profiling of the gut microbiome in HIV infection

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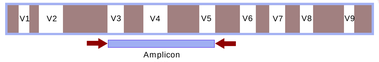
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INTRODUCTION

The gut microbiome is composed by ~14^{14} microorganisms that carry ~x100 fold human genes. It plays an essential role in human physiology including the development of the immune system, the homeostasis maintenance and the production of essential metabolites. The gastrointestinal (GI) tract also contains the largest immune compartiment in the body, the gut associated lymphoid tissue (GALT), which is the first point of contact with the HIV-1. The GALT of HIV-infected patients undergoes a severe and rapid deterioriation, and one of its consequences is a harsh microbial imbalance, also called dysbiosis. Compositional shifts in the microbiota inhabiting GI tract have been linked to health disorders. Here we aim to explore how HIV infection might influence the gut microbiome functions.

The Bacterial Barcode

The 16S small subunit ribosomal RNA (rRNA) gene is found in all prokaryotes. It contains nine hypervariable regions (white) flanked by sequences highly conserved among all prokaryotes (grey). DNA sequencing based on 16S rRNA genes allows for genus- and even species-level identification within a mixed microbial community such the gut microbiome.



Phenotype	N	ART	CD4+	HIV-1 VL
Late Presenters (LP)	5	No	≤ 200	-
Elite Controllers (EC)	3	No	-	< 50 x 1 year
Viremic Controllers (VC)	6	No	-	50-2000 x 1 year
ART-Naïve (N)	7	No	≥ 500	> 2000
Early Treated (ET)	5	Yes, < 6 months	-	< 50
Immune Concordant (IC)	27	Yes, > 2 years	≥ 500	< 50
Immune Discordant (ID)	11	Yes, > 2 years	≤ 300	< 50
HIV-negative Controls (Neg)	16	-	-	-

STUDY DESIGN

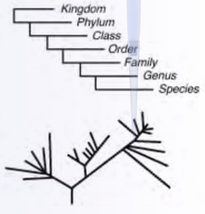
A cross-sectional study was designed including 80 subjects: 58 men, 21 women and 1 trasgender woman. All subjects were classified into different phenotypes according to three parameters: antiretroviral treatment (ART), CD4+ T cell count and viral load (VL). See abstract number 261 for a complete description of the groups.

MATERIALS AND METHODS

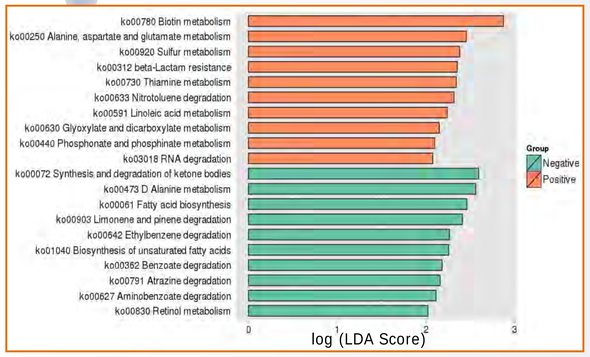
Total DNA was isolated from fecal samples of 80 individuals. Bacterial 16S rRNA genes were amplified by PCR with primers encompassing V3, V4 and V5 regions. The 16S rRNA amplicons obtained were sequenced using Illumina MiSeq.

16S rRNA amplicon sequences were filtered by length and quality with Trimmomatic. A minimum similarity threshold of 97% was set to cluster nearly identical filtered sequences into operational taxonomic units (OTUs) using mothur. These OTUs were then aligned against GreenGenes database (version May 2012) and classified accross multiple taxonomic levels, obtaining a bacterial content table.

PICRUSt was used to predict gene abundance of each sample from the bacterial content table. Gene content table was analyzed with HUMAnN to reconstruct the metabolic pathways and modules. Finally, we ran the LefSe algorithm to identify which pathways significantly contributed to differences among HIV states or phenotypes.



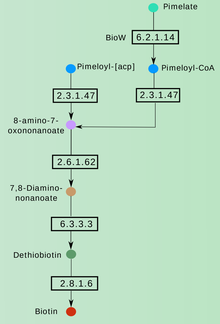
RESULTS AND DISCUSSION

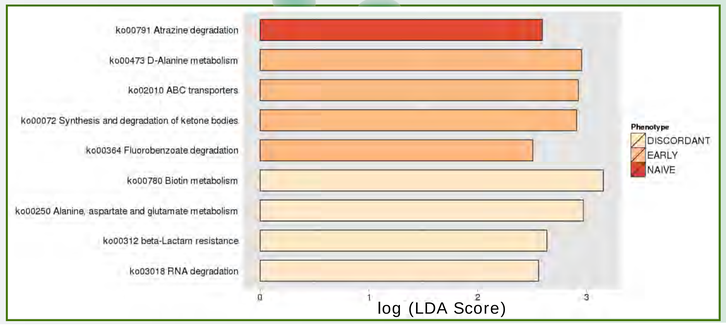


Most differences among HIV+ men involved the discordant (ID) and the early treated (ET) groups. The microbiome of ID patients was enriched for genes related to biotin metabolism; Ala, Asp and Glu metabolism; beta-lactam resistance and RNA degradation. The microbiome of ET patients contained more genes involved in D-Ala metabolism, synthesis and degradation of ketone bodies, fluorobenzoate degradation and ABC transporters, whereas naive subjects contained in their guts more bacterial genes associated to atrazine degradation.

Multiple significant differences were found when comparing the microbiome composition between men and women (data not shown). Consequently, we decided to include only males in downstream analyses. Within men, the microbiome of HIV positive subjects (5 LP, 1 EC, 3 VC, 6 AN, 5 ET, 17 IC and 8 ID) showed enrichment in genes implicated in biotin metabolism; ala, asp and glu metabolism; sulfur metabolism; beta-lactam resistance; thiamine metabolism, nitrotoluene degradation; linoleic acid metabolism; glyoxilate and dicarboxylate metabolism; phosphonate and phosphinate metabolism and RNA degradation relative to HIV negative (N=13). The gut microbiome of HIV negative subjects had more genes associated to synthesis and degradation of ketone bodies, ala metabolism and fatty acid biosynthesis, among others.

Biotin metabolism was one of the most significant biomarkers that contributed to the differentiation among HIV status or phenotypes. Biotin is an essential cofactor of important enzymes that participate in multiple metabolic pathways. Mammals obtain biotin from commensal bacteria, which synthesize it from pimelate thioester through the activity of 5 enzimes. It has been reported that biotin regulates TNF-a production, which plays important roles in the pathogenesis of inflammatory diseases.





CONCLUSIONS

HIV infection is associated with bidirectional unbalances in intestinal metabolic activity affecting mucosal barrier integrity and function, local and systemic inflammatory processes and energy storage and consumption.