Complexities of Gut Microbiome Dysbiosis in HIV-Infected and High-Risk Populations

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Background

In the gut of healthy individuals, there is important communication between the immune system and the microbiota that helps keep both systems at homeostasis. However, in HIV infection, the virus targets activated CD4+ T cells resulting in their massive depletion as well as compositional alteration of immune cells in the gut. These effects are incompletely restored after antiretroviral therapy (ART). Because the gut innate and adaptive immune systems and a healthy gut epithelium are critical for maintaining a healthy gut microbiome, this sustained immune dysfunction may be a driving factor of sustained dysbiosis. The attributes of the gut microbiome in the context of HIV infection and ART are important to understand, as increased immune activation driven by translocation of gut bacterial components has been linked to disease progression in both untreated and treated infection. Gut microbiome composition in HIV-infected populations has been shown by our lab and others to be significantly different from that of HIV negative individuals, even with effective ART[1-5]. However, subsequent studies[6] have shown that sexual preference in Men who have Sex with Men (MSM) is an important factor in shaping gut microbiome composition, and a driving factor of many differences often attributed to HIV infection itself. Here, we use 16S rRNA sequencing of feces of a large cohort to further define MSM-associated microbiome differences and differences associated with HIV and ART in MSM and women.

Methods

16S rRNA targeted sequencing of feces was performed on our cohort of 217 individuals. The analyses controlled for sexual behavior, and gender to further characterize gut microbiome attributes that are associated with HIV infection and ART, and show unique HIV-associated changes in MSM versus women.

Table 1. Cohort Description

		N	Age (years)	IVDU	CD4 (cells/uL)	Viral Load (copies/mL)
HIV Negative	MSM	35	34.5 +/- 8.5	0	NA	0
	MSW	29	38.0 +/- 14.3	0	NA	0
	Women	41	32.9 +/- 9.9	0	NA	0
HIV Positive ART Naïve	MSM	40	33.5 +/- 9.1	2 (5.0%)	560 +/- 206 (201-1095)	126714 +/- 189725 (159-856000)
	MSW	1	51.0	0	551	3880
	Women	4	30.0 +/- 5.2	1 (25.0%)	600 +/- 357 (103-1071)	33250 +/- 40880 (7000-104000)
HIV Positive ART Treated	MSM	50	50.0 +/- 10.1	1 (2.0%)	699 +/- 311 (177-1297)	12 +/- 14 (0-44)
	MSW	2	55.5 +/- 1.5	0	926 +/- 344 (619-1306)	10 +/- 10 (0-20)
	Women	15	50.9 +/- 9.4	0	816 +/- 336 (187-1417)	35 +/- 80 (0-297)

Results

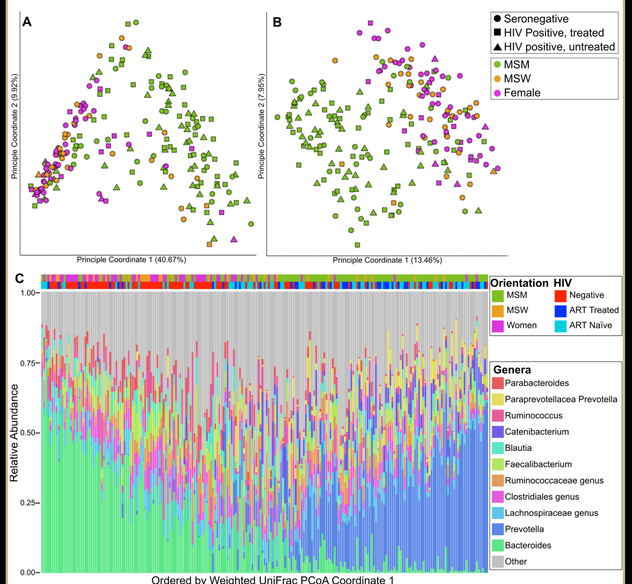


Figure 1. MSM is strongly associated with Prevotella richness; HIV infection is not. A. Weighted Unifrac PCoA and **B.** unweighted UniFrac with points colored by orientation and shaped by HIV status. **C.** Genus level taxonomic summary plots ordered by weighted Unifrac PCoA Coordinate 1. Genera with a mean relative abundance of less than 1% are binned together as Other.

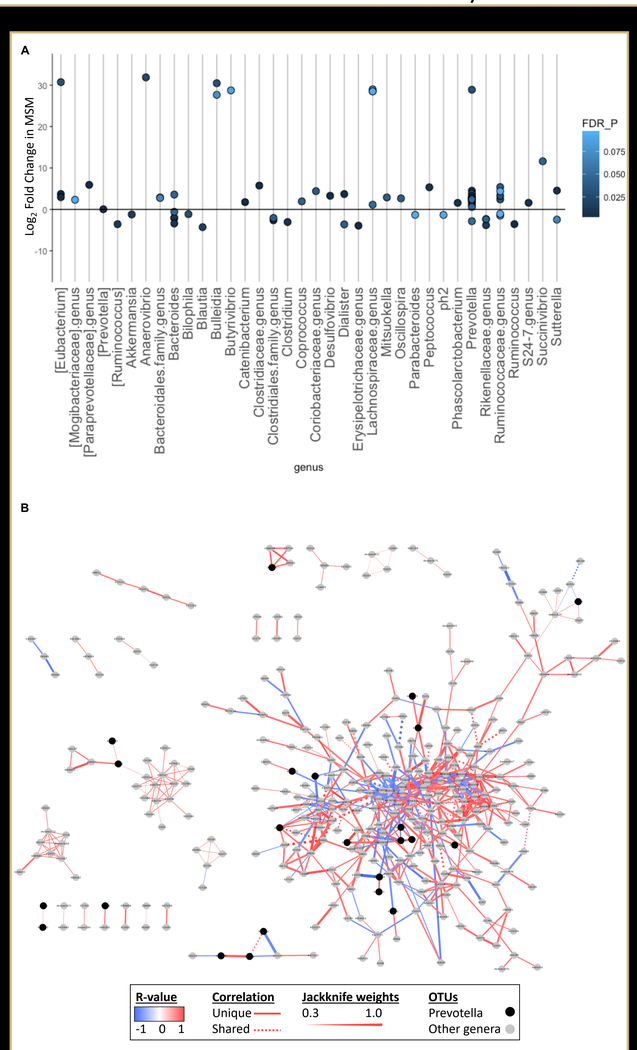


Figure 2. MSM-associated Prevotella enterotype significantly differs from non-MSM Prevotella enterotype in both abundance and correlations of OTUs. A. Log_2 fold change of relative abundance of significantly altered OTUs between MSM and non-MSM binned by genus with points colored by FDR P-value. The subset of individuals within the Prevotella enterotype space included 105 MSM and 27 non-MSM (MSW and women). Within this subset 71 OTUs had significantly different relative abundance between MSM and non-MSM (FDR P < 0.1, Mann-Whitney U test). **B.** Correlation network of OTUs in HIV-negative, Prevotella-rich MSM. Correlation network of the 345 OTUs in the subset of 25 individuals calculated using SparCC. Value of the correlation is colored on the network edges with negative R-values in blue and positive in red. Only correlations greater than |0.5| are included in the network. Analysis was jackknifed 100 times on a random subset of 9 individuals. The edge weights from the jackknife are represented by line weight, with the thickest lines corresponding to correlations identified in all jackknife subsets. The network edges on this graph are compared to a network calculated using the same methods with a subset of 9 individuals that are HIV-negative, Prevotella-rich non-MSM. The edges that are shared between the MSM and non-MSM networks are dotted and the edges unique to the MSM network are solid. Lastly, the OTUs identified in the Prevotella genus are colored black to aid in visualization that 93% of the edges containing one or more Prevotella nodes are unique to the MSM network.

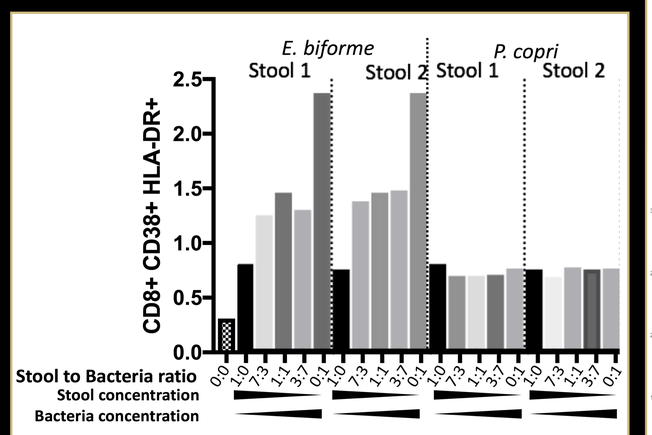


Figure 3. Some enriched components of the MSM Prevotella enterotype (*Eubacterium biforme*) but not *Prevotella copri* activate CD8+ T cells. CD8+ CD38+ HLA-DR+ cell percent from peripheral blood monocytes (PBMCs) cultured with various ratios of stool bacteria spiked with either *E. biforme* or *P.copri*. A large increase in activated CD8 T cells was observed with *E. biforme* and not with *P. copri,* suggesting that inflammatory species/strains associated with the MSM Prevotella enterotype and not the Prevotella richness itself may influence increased gut inflammation.

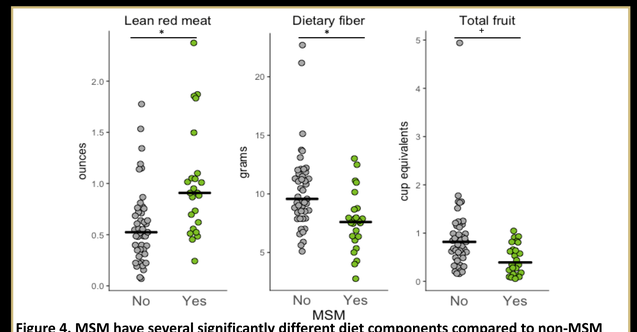


Figure 4. MSM have several significantly different diet components compared to non-MSM that does not follow expected trends in Prevotella-rich microbiomes. Food frequency questionnaires were give to 107 of study subjects at time of sample collection. Meat and fruit consumption reported based on daily servings, fiber is reported from total consumption. (Kruskal-Wallace test; FDR p-value: + < 0.1, * < 0.05)

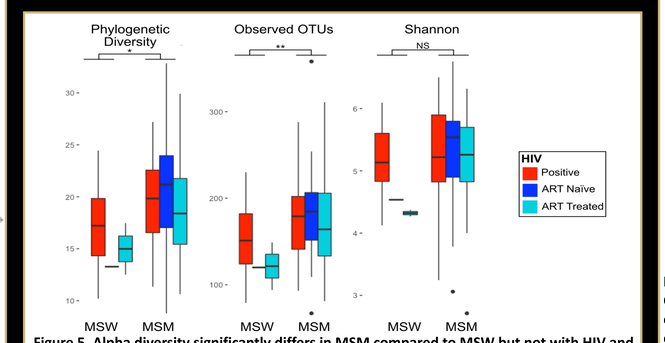


Figure 5. Alpha diversity significantly differs in MSM compared to MSW but not with HIV and ART. Alpha diversity in men significantly increases in MSM compared to MSW but no significant trend found with HIV or ART (Kruskal-Wallace test; p-value: * < 0.05, ** < 0.01)

Table 2. In MSM individuals there are several significantly different OTUs in HIV and ART that follow two significant patterns. Significant OTUs determined by Kruskal-Wallace test (listed P-values), patterns determined by Dunn's post-hoc test (difference defined by FDR P < 0.1)

	MSM				
	Mean Relative Abundance			FDR	
Taxonomy	HIV Negative	Untreated	Treated	P-value	P-value
No difference in HIV and significant reduction in ART treatment (¯\ )					
Fenollaria massiliensis	1.76E-03	2.00E-03	3.01E-04	<0.001	<0.05
Peptoniphilus sp.	1.44E-03	2.41E-03	5.28E-04	<0.001	<0.05
Significant increase in HIV and significant reduction in ART treatment (/\ )					
Streptococcus sp.	5.09E-05	1.84E-03	2.32E-05	<0.001	<0.01
Fusobacterium equinum	1.17E-03	7.77E-03	1.84E-04	<0.001	<0.05
Turicibacter sanguinis	5.55E-04	6.02E-04	3.17E-04	<0.01	<0.1
Finegoldia magna	5.60E-03	8.26E-03	5.35E-03	<0.01	<0.1

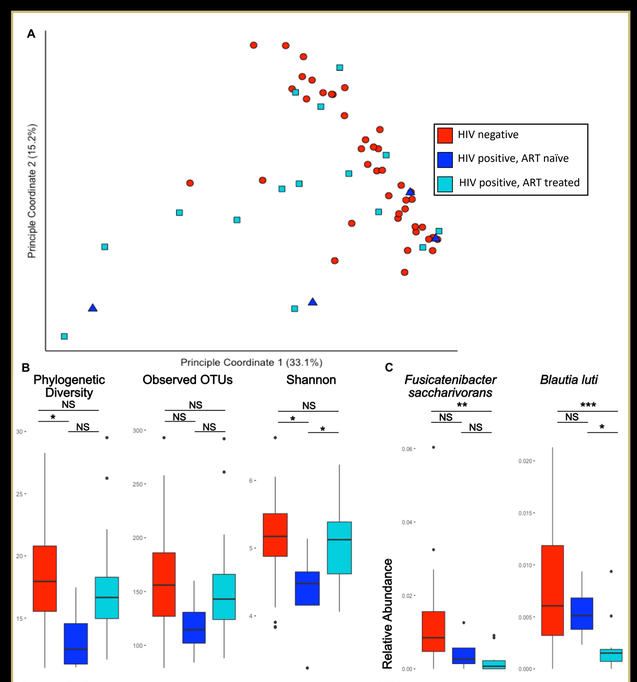


Figure 6. Despite compositional changes in only half of HIV positive women, there are two significantly different OTUs in HIV and ART that follow two significant patterns and HIV positive, untreated women have significantly reduced alpha diversity. A. Weighted UniFrac PCoA of women only shows that only half of HIV-positive women cluster apart from HIV-negative women. **B.** Alpha diversity measures of women only show significant reduction in HIV positive, untreated women **C.** Two OTUs significantly reduced in HIV-positive, treated women. (Kruskal-Wallis, Dunn's post hoc test; FDR p-value: * < 0.05, ** < 0.01, *** < 0.001)

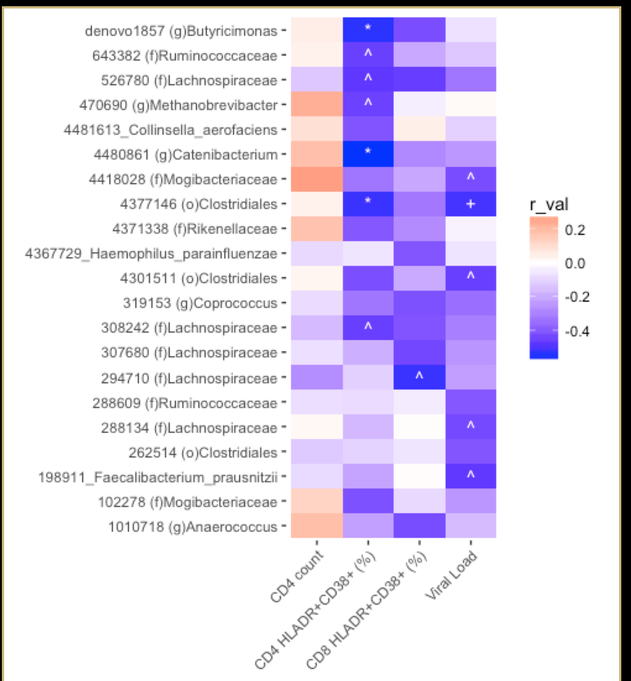


Figure 7. Several OTUs negatively associated with immune activation in untreated MSM. Correlations in HIV-positive, untreated MSM between OTUs and serum measures of CD4+ T cell count, CD4+/CD8+ CD38+ HLA-DR+ cell percent, and viral load. Several OTUs are negatively correlated with these markers of immune activation/disease progression, suggesting the microbiome composition may influence disease progress in untreated infection. (Spearman Rank test; FDR p-value: ^ < 0.2, + < 0.1, * < 0.05)

Conclusions

- We confirmed previous reports that Prevotella-rich microbiomes with increased alpha diversity are strongly associated with men who have sex with men (MSM) and not HIV.
- Analysis with HIV-negative Prevotella-rich individuals revealed significantly different microbiome composition and microbial co-occurrence in MSM compared to non-MSM.
- MSM are eating more red meat and less fruit and fiber than non-MSM, the opposite diet than previously reported in population with Prevotella-rich microbiomes.[7]
- Analysis in women only as well as in MSM show significant alterations in microbiome taxa in treated and untreated HIV infection.
- Several taxa changes may be associated specifically with ART usage.
- Analyses of immune activation in ART-naïve HIV-infected individuals revealed several taxa associated with decreased immune activation and viral load.

This study provides an in-depth characterization of microbiome differences that occur in a US population infected with HIV and the degree to which these differences may be driven by lifestyle factors, ART, and HIV infection itself. This understanding will help to guide efforts to investigate the functional implications of these differences to ultimately target the microbiome to improve the health of this population.

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