

Male Genital Tract Microbiota: Insights into HIV Acquisition

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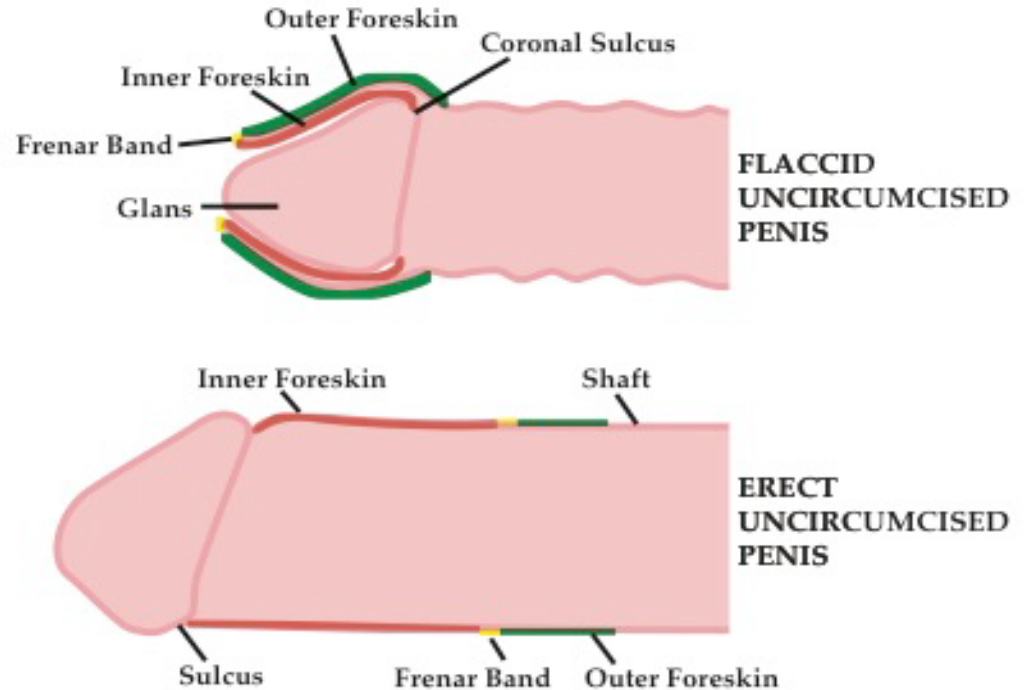
Johns Hopkins University



Male Circumcision

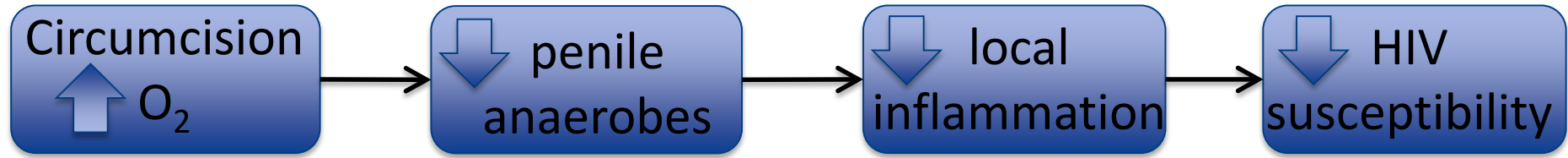
Circumcision
reduces HIV-1
acquisition by 60%

Still don't really
understand how





Hypothesis



- BV in women → Genital anaerobes = HIV susceptibility
- Mediated by inflammation?

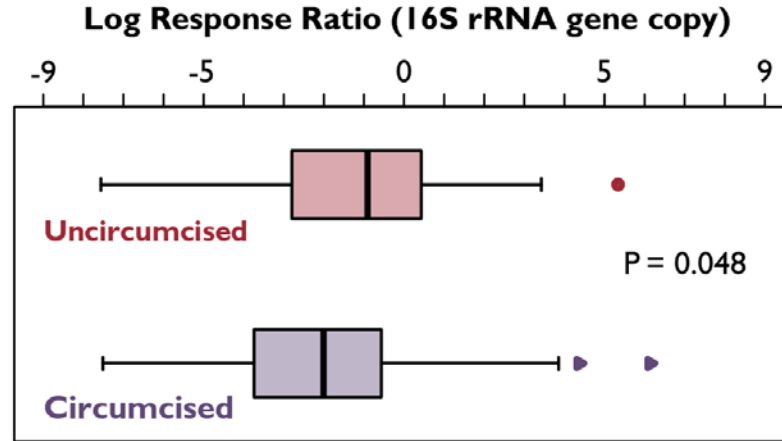


Methods

- Swab samples of coronal sulcus (microbiome, soluble immune markers)
- Foreskin tissue collection (model for mucosal immune responses)
- Microbiome
 - 16S rRNA gene-based quantitative PCR and pyrosequencing
- Soluble chemokines
 - Multiplexed ELISA (MesoScale Discovery)



Circumcision reduces prevalence and load of penile anaerobic bacteria

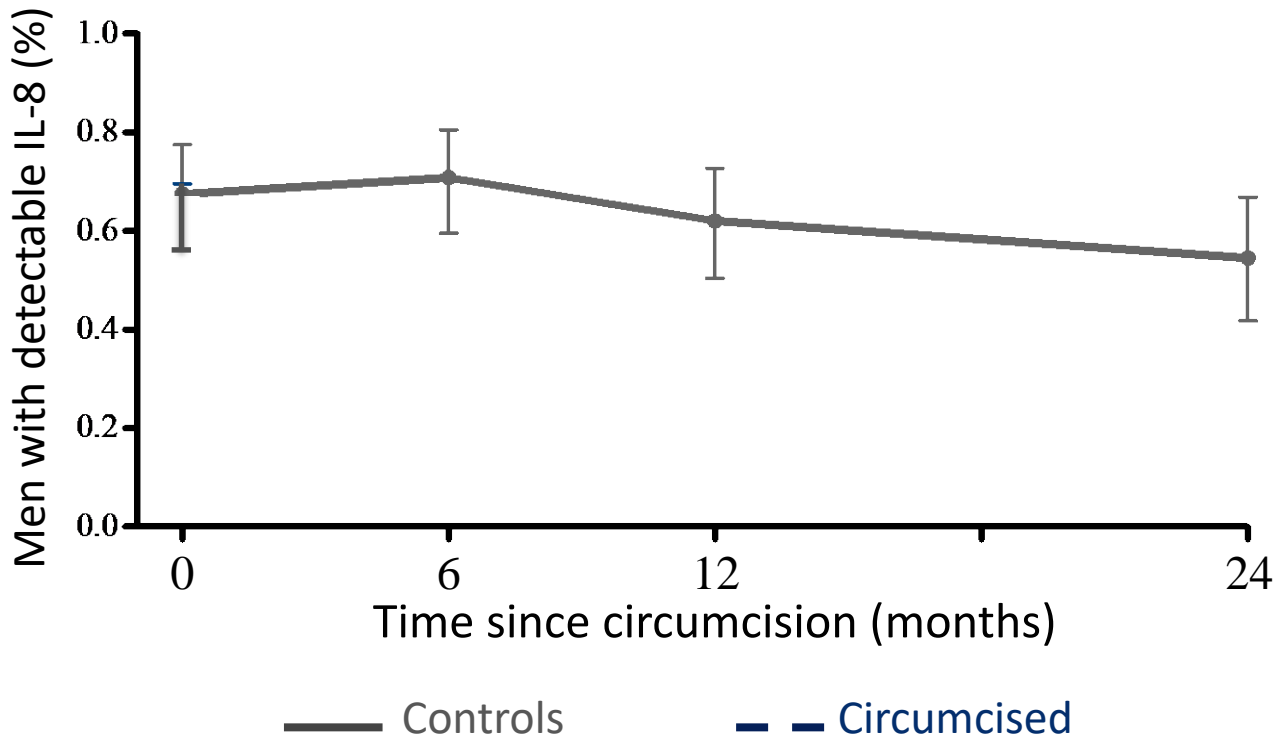


- MC decreased total bacterial load
- MC decreased microbiota diversity
 - Slight increase in aerobic taxa
 - Prevalence and absolute abundance of 12 bacterial taxa significantly decreased

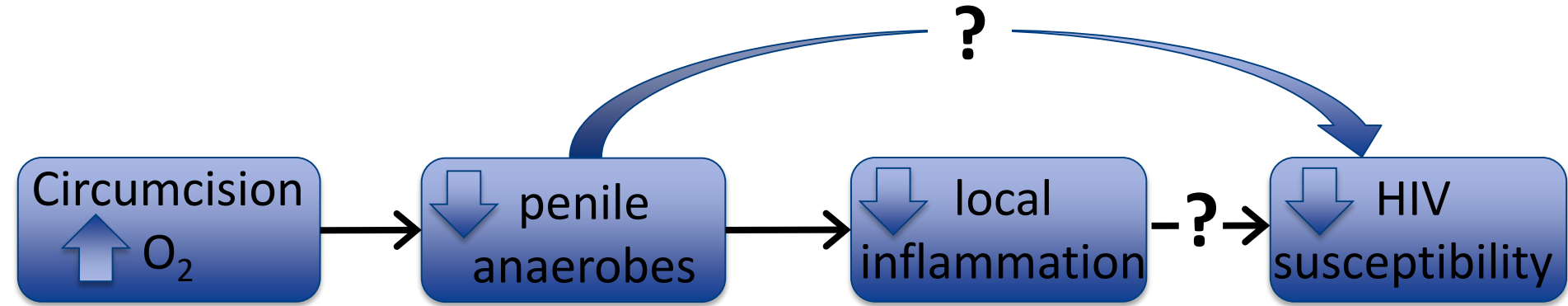


Circumcision decreases penile IL-8

	% Detectable
IL-8	59.4
MIG	25.0
GM-CSF	6.7
MCP-1	6.7
MIP3 α	5.0
IL-1a	3.9
RANTES	2.8



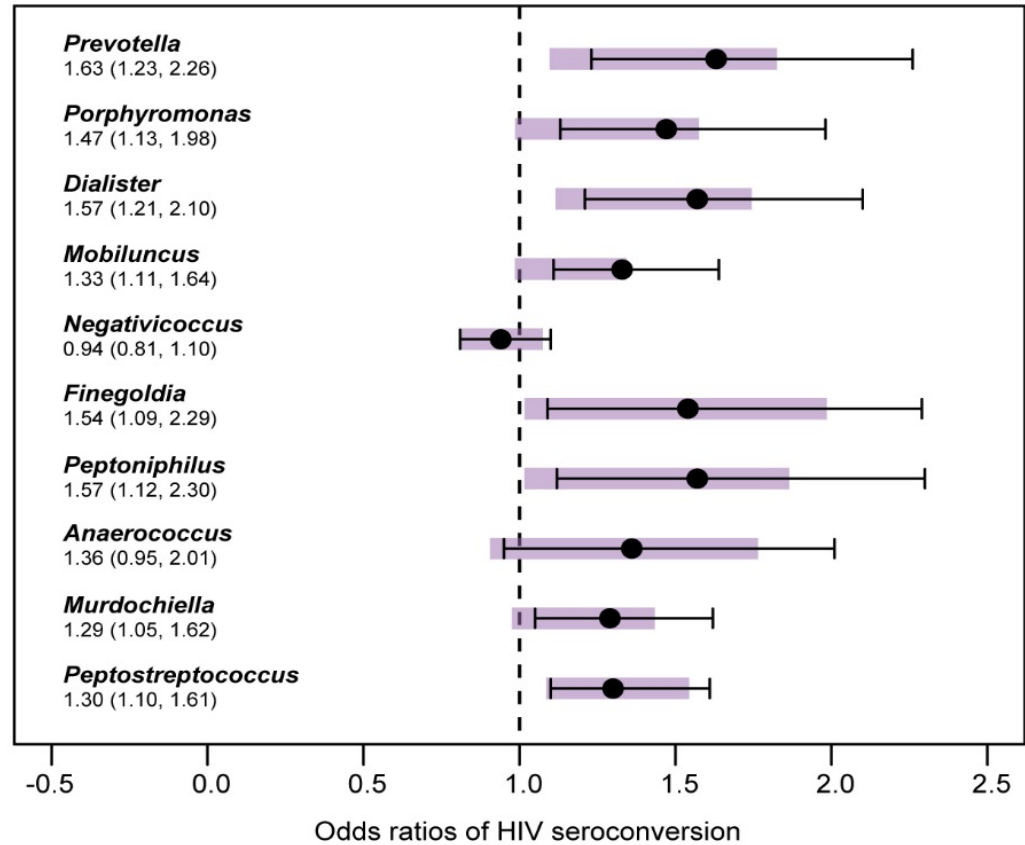
Testing our hypothesis:



- Nested case-control study of seroconverters:
 - 60 seroconverters, 120 control (all uncircumcised)



Higher absolute abundance of anaerobic bacteria associated with seroconversion



- Remains significant after controlling for STIs and other demographics

Cytokines associated with seroconversion

Detectable Cytokine	Controls n=120	Seroconverters N=60	OR (95% CI)	aOR (95% CI)
IL-8	63 (52.5%)	44 (73.3%)	2.52 (1.28, 4.99)	2.26 (1.04, 6.40)
MIG	23 (19.7%)	22 (36.7%)	2.49 (1.23, 5.03)	2.72 (1.15, 8.06)
GM-CSF	5 (4.2%)	7 (11.7%)	3.02 (0.92, 9.91)	
MCP-1	6 (5.0%)	6 (10.0%)	2.10 (0.65, 6.79)	
MIP3 α	4 (3.3%)	5 (8.3%)	2.61 (0.68, 10.06)	
IL-1a	4 (3.3%)	3 (5.0%)	1.53 (0.33, 7.16)	
RANTES	3 (2.5%)	2 (3.3%)	1.35 (0.22, 8.30)	

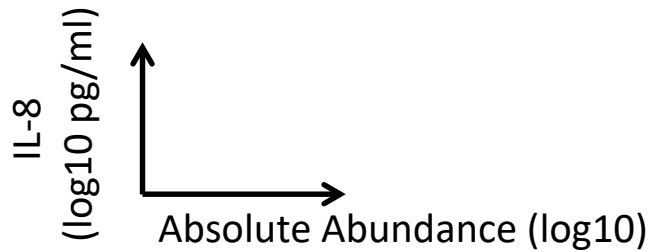
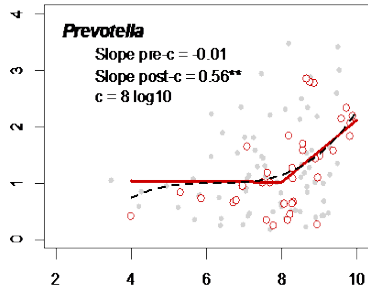
^A Adjusted for STI diagnostics (syphilis and HSV-2), and all variables associated with either seroconversion, IL-8 or MIG (occupation, marital status, multiple sex partners, condom use, alcohol use, STI symptoms)

Cytokines associated with seroconversion

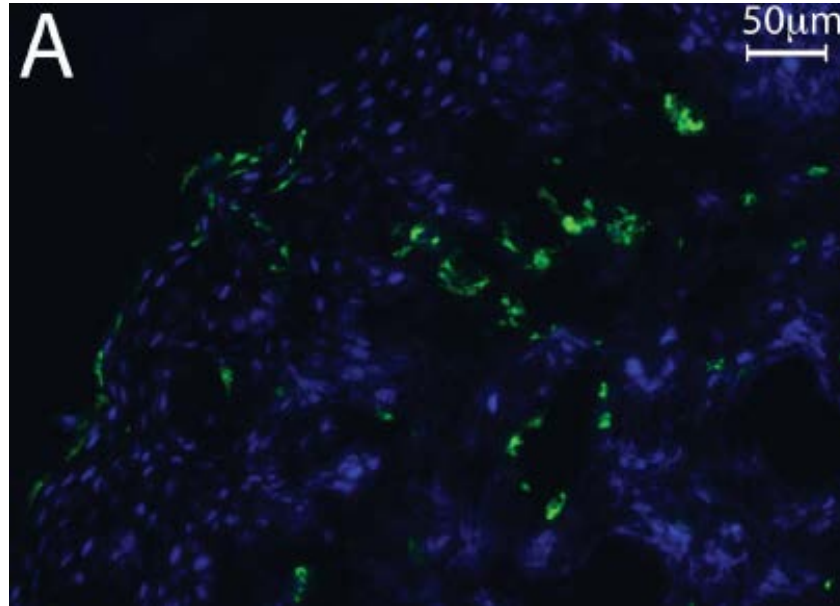
Detectable Cytokines	Controls n=120	Seroconverters N=60	OR (95% CI)	aOR (95% CI)
0	55 (45.8%)	14 (23.3%)	REF	REF
1	42 (35.0%)	25 (41.7%)	2.34 (1.09, 5.03)	2.56 (0.93, 7.70)
2+	23 (19.2%)	21 (35.0%)	3.78 (1.61, 8.90)	3.30 (1.21, 12.50)

^A Adjusted for STI diagnostics (syphilis and HSV-2), and all variables associated with either seroconversion, IL-8 or MIG (occupation, marital status, multiple sex partners, condom use, alcohol use, STI symptoms).

Anaerobes and IL-8



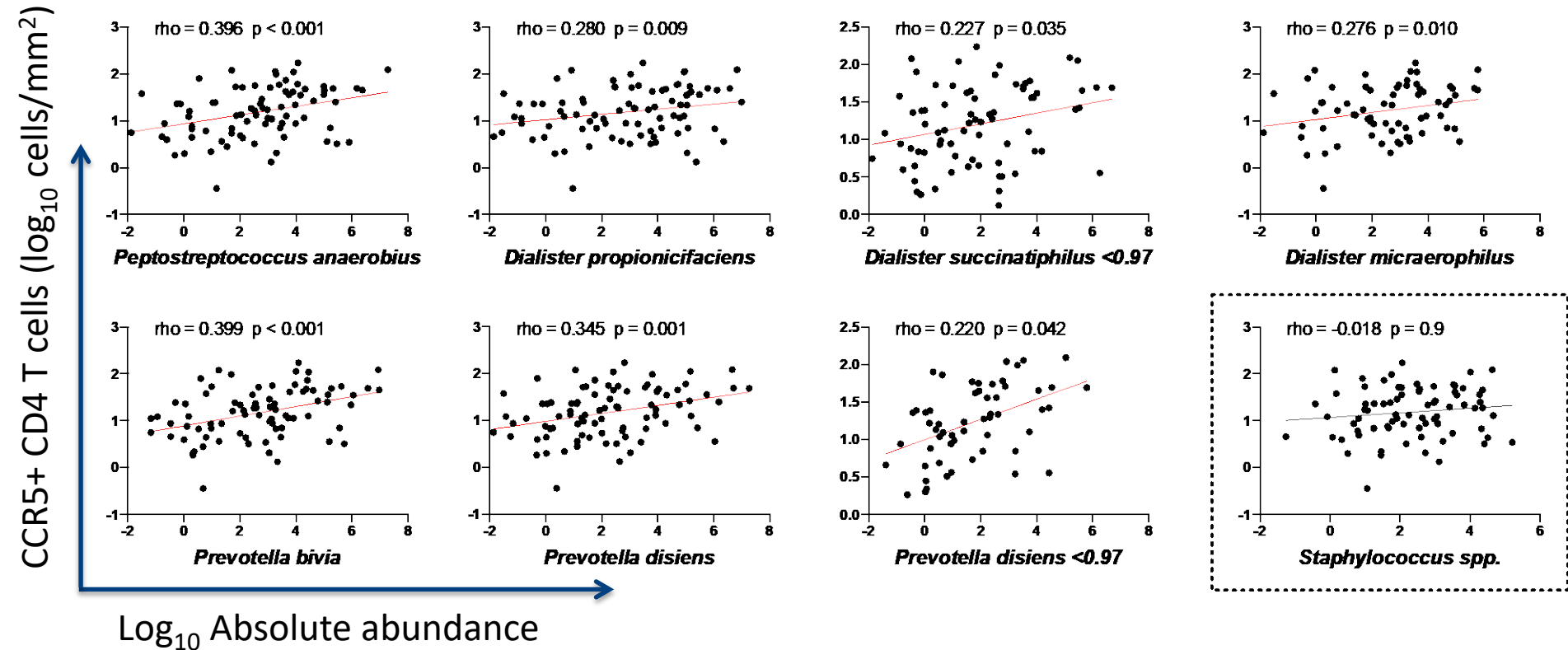
IL-8 and MIG Associated with Increased Neutrophils in Foreskin



CD15+ neutrophils in the foreskin highlighted in green by immunofluorescence

Prodger 2016 Plos Path

Anaerobe species correlate with HIV target cells



Summary

- Male circumcision reduces penile anaerobes & IL-8
- Increased penile cytokines are associated with subsequent HIV seroconversion
- Penile anaerobes associated with increased penile IL-8 levels
- IL-8 associated with increased density of foreskin CCR5+ CD4 T cells and neutrophils in the foreskin
- Circumcision may protect against HIV by reducing local immune activation through the elimination of key anaerobes

Conclusions and Future Directions

- The foreskin provides a unique opportunity to understand mucosal immunology and HIV acquisition.
- Further understanding of penile inflammation is needed to develop novel mechanisms to reduce HIV acquisition and transmission.
 - Using discovery-based analysis, evaluating what penile bacterial species associated with seroconversion
 - Assessing whether reproductive maturation and sexual debut in adolescent boys leads to changes in the penile microbiome that affects the genital immunological milieu and risk of acquiring HIV

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