Pregnancy associated vaginal proteome alterations linked to HIV acquisition risk

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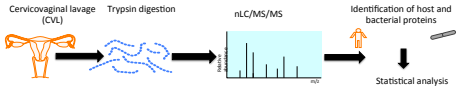
Background

- HIV acquisition risk increases two-fold during pregnancy[1]
- In non-pregnant women, HIV-acquisition is associated with:
  - mucosal inflammation of the genital tract
  - concurrent sexually transmitted infections
  - certain hormonal contraceptives
  - non-*Lactobacillus* dominant microbiome/ vaginal dysbiosis[2]
- However, pregnancy is generally associated with a more stable and *Lactobacillus*-dominant microbiome, yet the risk of HIV-acquisition is increased[3]
- We performed proteomic analysis on samples from 23 pregnant and 25 non-pregnant women to try to understand the etiology of the increased risk of HIV-acquisition in pregnancy

Methods



Cervicovaginal lavage (CVL) samples were collected from 23 pregnant and 25 non-pregnant women from an Obstetrics and Gynecology Clinic in Los Angeles, California and were analyzed by mass spectrometry. Bacterial proteins were identified from a curated TrEMBL database as published in Klatt *et al.*, 2017, Science. Host proteome data base matched to the curated SwissProt Human database as described previously in Birse et al., JID, 2017. Bacterial functions were annotated using the KEGG ontology database. Enrichment analysis of pregnancy signatures to other HIV risk cohorts (Abdool Karim et al., Science, 2010) were performed using GSEA

Cohort

Variable	Variable category	Pregnant women	Non-pregnant women	p value
		n=23	n=25	
Age (Mean ± SD; range)		27.8±5.8; (17-38)	33.3±7.3; (19-44)	0.02*
Race (n,%)¥	Hispanic/Latina	21 (91.3)	24 (96.0)	0.601
	Non-Hispanic Black	1 (4.3)	0 (0)	
	Non-Hispanic White	1 (4.3)	0 (0)	
	Asian	0 (0)	1 (4.0)	
Ectopy (n, %)	Without ectopy	9 (39.1)	15 (60.0)	0.728
	With ectopy	14 (60.9)	10 (40.0)	
Cervical percentage of ectopic area (Mean±SD; range)		41.9±15.8; (11.2-62.1)	16.7±16.1; (0.1-49.3)	0.000 2*
Gestation Days (Mean ±SD; range)		180.4±50.0 (99-259)	N/A	
Parity	0 previous births	10 (43.5%)	7 (30.4%)	0.33
	1-2 previous births	7 (30.4%)	9 (39.1%)	
	3+ previous births	6 (26.1%0	7 (30.4%)	
Last coitus	Past 7 days	13 (56.5%)	16 (60.0%)	0.700
	Past month	5 (21.7%)	6 (24.0%)	
	Past year	5 (21.7%)	3 (12.0%)	

¥: p value is from Fisher's exact test, where non-Hispanic black, non-Hispanic white and Asian were combined
*: Statistically significant (p<0.05)

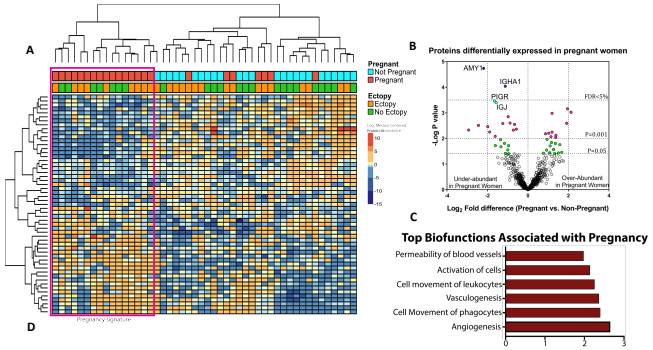
References

1) Gray R. H. Lancet 2005; 366:1182-8
2) Vitali, D. AIDS Res Ther 2017; 14:39
3) Romero R. Microbiome 2014; 2:4
4) Klatt N. Science 2017; 356: 938-45
5) Birse K. JID 2017; 215:590-8
6) Abdool Karim Q. Science 2010; 329: 1168-74

Results – Host Proteome

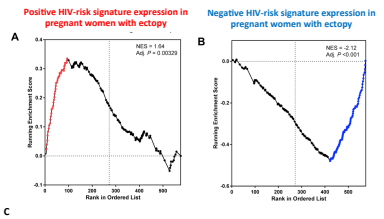
Figure 1: Pregnancy proteome alterations in CVL were immunosuppressive

MS analysis of CVL samples collected from pregnant and non-pregnant women identified 506 human proteins., of which 56 (10%) were differentially abundant (P<0.05). **(A)** Differentially expressed proteins were analyzed by unbiased hierarchical clustering. A proteomic-defined pregnancy signature (pink box) was defined by 27 overabundant and 29 underabundant proteins in pregnant women. **(B)** A volcano plot of all proteins identified comparing pregnant and non-pregnant women. **(C)** Biofunctional associations with the Pregnancy Signature were determined by using Ingenuity Pathway Analysis (p<0.05) and identified activated inflammatory and blood vessel formation pathways. (D) Biofunctional analysis using DAVID gene ontology annotations identified complement components and leukocyte recruitment factors as a part of the Pregnancy Signature.



Altered Pregnancy Pathways	p value	Associated Proteins
Complement system	3.63×10^{-3}	SERPING1 ↓, CF1 ↓
Leukocyte extravasation signaling	1.45×10^{-2}	TIMP1 ↓, TIMP2 ↓, RAP1A ↑

Figure 2: Pregnancy/ectopy proteome signature overlap with CAPRISA risk signature for HIV acquisition



Gene Set Enrichment Analysis (GSEA) was used to analyze the overlap between the Pregnancy Signature and proteomic signatures identified in women at high risk for HIV acquisition. HIV risk signatures were derived from cervicovaginal fluid proteins differentially expressed at pre-seroconversion time points in women who acquired HIV within the CAPRISA 004 trial relative to HIV negative controls (Noël-Romas *et al.* CROI 2018, poster #271). The strongest overlap was observed in pregnant women with cervical ectopy. GSEA walking plots show the running enrichment score of proteins that were **(A)** overabundant or **(B)** underabundant. **(C)** All GSEA results are summarized. Analysis of all pregnant women did not significantly overlap with the HIV risk signature, only pregnant women with ectopy showed significant enrichment (NES > |1.5|, adj. P<0.05). NES=Normalized Enrichment Score.

Gene Set ID	Risk Signature Overlap	Participants included	No. Leading Edge Proteins	NES	FDR adj. p value
All Pregnant vs .Not Pregnant	Positive	All	66	-1.35	0.044
All Pregnant vs .Not Pregnant	Negative	All	54	-0.66	0.996
Pregnant Ectopy vs .No Ectopy	Positive	All pregnant	42	1.64	0.003
Pregnant Ectopy vs .No Ectopy	Negative	All pregnant	80	-2.12	<0.001

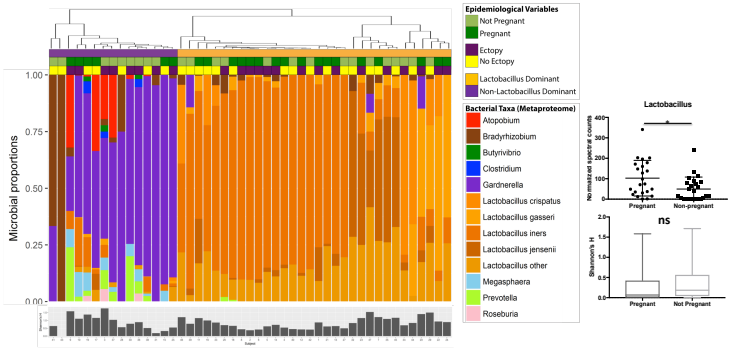
Conclusions

- Pregnancy is associated with changes to mucosal proteome pathways
  - immune system depression
  - increased blood vessel formation
  - decreased mucosal barrier function
- Some of the changes during pregnancy are similar to those observed in women who subsequenstly acquired HIV-infection
- Microbial metabolic pathways for carbohydrate metabolism and neutrophil function are increased during pregnancy

Results - Microbiome

Figure 3: Proteome alterations associated with ectopy corresponded to a non-Lactobacillus dominated microbiome

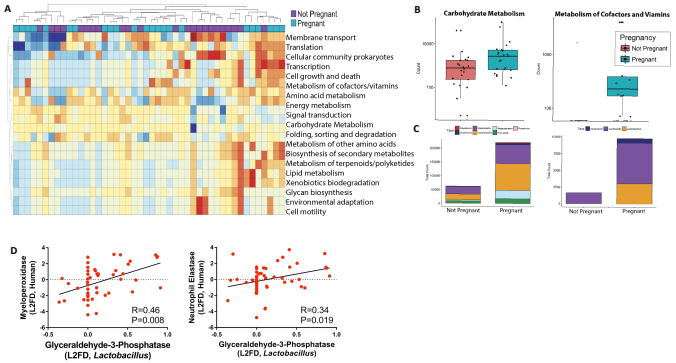
Metaproteomic analysis of CVL samples identified 376 bacterial proteins from 9 genera. The most abundant bacterial genera were *Lactobacillus*, *Gardnerella*, *Prevotella*, *Megasphaera* and *Atopobium*. **(A)** Women clustered predominantly into either a *Lactobacillus* dominant group (68%) or a non-*Lactobacillus* dominant group (32%). **(B)** Pregnant women had significantly higher levels of Lactobacillus-derived proteins than non-pregnant women. **(C)** There was no difference in microbial diversity between pregnant and non-pregnant women when evaluated by Shannon's diversity index.



Functional Microbiome

Figure 4: Pregnancy status was associated with alterations to bacterial metabolic functions

(A) Bacterial functional data for pregnant and non-pregnant women was obtained from KEGG ontology. **(B)** Statistically different functions between pregnant and non-pregnant women were determined using Wilcoxon Rank Sum Test and confirmed with bootstrapping and permutation analyses. Carbohydrate metabolism and Metabolism of Cofactors and Vitamins are increased in pregnant women. **(C)** Bacterial genera responsible for statistically different functions in pregnant and non-pregnant women. *Lactobacillus* and *Gardnerella* proteins largely produced the proteins involved in significantly differentially expressed microbial functions in pregnant women. **(D)** Spearman's rank correlations of host inflammatory proteins and metabolic proteins from *Lactobacillus*. Neutrophil factors significantly correlated with correlate with bacterial carbohydrate metabolism proteins.



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