METABOLITES, PRETERM LABOR AND ANTIRETROVIRAL THERAPY

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BACKGROUND

- HIV is associated with an increased risk of Preterm Birth
- Antiretroviral Therapy (ART), specifically protease inhibitors (PI), is also associated with an increased risk of Preterm Birth
- PI-associated Preterm Birth may be in part due to alterations in progesterone and estrodial metabolism[1,2]
- In the PROMISE Trial[3], the rates of Preterm Birth were
  - 13% on Zidovudine (ZDV) monotherapy versus
  - 20% on PI-based ART
- We sought to determine if untargeted metabolomics could help identify pathways associated with preterm birth and PI-associated Preterm Birth
- Study was performed as a proof-of concept of the metabolomics approach to detect signatures of Preterm Birth

METHODS

- A case-control study of 100 mother-infant dyads was performed on mothers who delivered preterm (<37 weeks gestation, n=50) or term (n=50)
- Maternal plasma and paired dried blood spots (DBS) were run from the timepoint closest, but prior to delivery in the preterm group and matched for gestational age at sampling in the term group
- The first infant DBS samples (birth or 1 week of age) were run for the infants
- Untargeted metabolomics using ultra-high performance liquid chromatography/tandom mass spectrometry was run by Metabolon ®, Morrisville, NC
- Linear regression (LR), random forests (RF) and LASSO modeling were performed to identify potential metabolic signatures of preterm birth

REFERENCES

1. Papp E et al. JID 2015
2. McDonald CR CID 2018
3. Fowler MG et al. NEJM 2016

ACKNOWLEDGEMENTS

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RESULTS

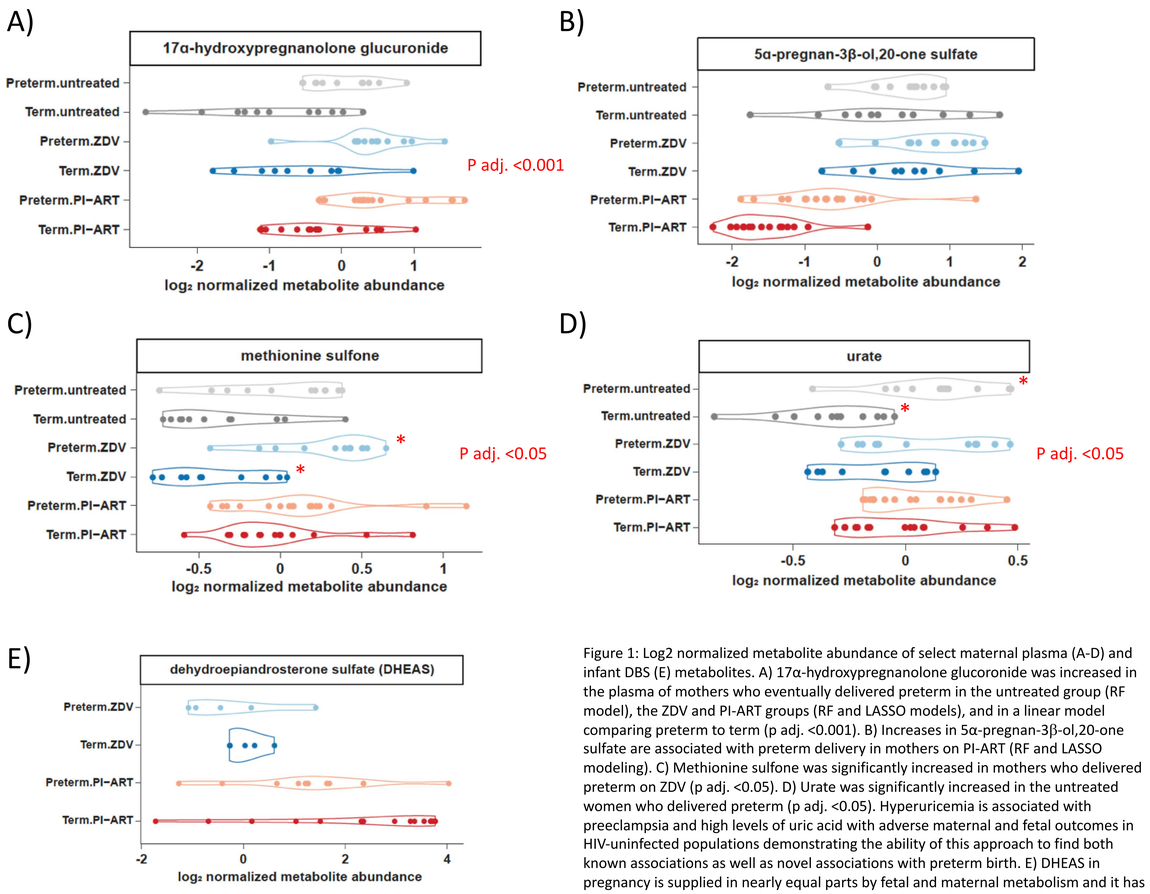


Figure 1: Log2 normalized metabolite abundance of select maternal plasma (A-D) and infant DBS (E) metabolites. A) 17α-hydroxypregnanolone glucoronide was increased in the plasma of mothers who eventually delivered preterm in the untreated group (RF model), the ZDV and PI-ART groups (RF and LASSO models), and in a linear model comparing preterm to term (p adj. <0.001). B) Increases in 5α-pregnan-3β-ol,20-one sulfate are associated with preterm delivery in mothers on PI-ART (RF and LASSO modeling). C) Methionine sulfone was significantly increased in mothers who delivered preterm on ZDV (p adj. <0.05). D) Urate was significantly increased in the untreated women who delivered preterm (p adj. <0.05). Hyperuricemia is associated with preeclampsia and high levels of uric acid with adverse maternal and fetal outcomes in HIV-uninfected populations demonstrating the ability of this approach to find both known associations as well as novel associations with preterm birth. E) DHEAS in pregnancy is supplied in nearly equal parts by fetal and maternal metabolism and it has been suggested that perturbations of DHEAS production by protease inhibitors may contribute to adverse infant outcomes.

CLINICAL CHARACTERISTICS

Table 1: Characteristics and ART exposure at time of maternal sampling

	Preterm				Term				P-Value*
Treatment	Untreated	ZDV	PI-ART	All	Untreated	ZDV	PI-ART	All	
N	13	18	18	50	15	13	20	50	
Male Gender	5 (39)	6 (33)	8 (44)	20 (40)	6 (40)	6 (46)	11 (55)	24 (48)	0.546
Birth Weight (g)	2643 (499)	2584 (530)	2453(470)	2555(490)	2933(329)	2811(482)	2830(552)	2877 (472)	0.002
Country									1
India	0 (0)	0 (0)	2 (11)	2 (4)	1 (7)	0 (0)	1 (5)	2 (4)	
Malawi	5 (39)	13 (72)	6 (33)	24 (48)	8 (53)	10 (77)	5 (25)	24 (48)	
South Africa	6 (46)	4 (22)	8 (44)	19 (38)	6 (40)	2 (15)	10 (50)	19 (38)	
Uganda	2 (15)	1 (6)	1 (6)	4 (8)	0 (0)	1 (8)	3 (15)	4 (8)	
Zambia	0 (0)	0 (0)	1 (6)	1 (2)	0 (0)	0 (0)	1 (5)	1 (2)	
GA at Collection	29.8 (2.8)	30.8 (2.7)	31.2 (2.3)	30.7 (2.6)	31.0 (2.8)	30.8 (2.5)	30.8 (2.4)	30.8 (2.7)	0.927
GA at Delivery	32.0 (3.1)	33.2 (2.5)	33.5 (2.)	33.1 (2.5)	40.6 (3.9)	39.7 (2.4)	39.8 (2.1)	40.0 (2.8)	<0.001
Days PTD				16.5 (9.9)				64.6 (22.3)	<0.001

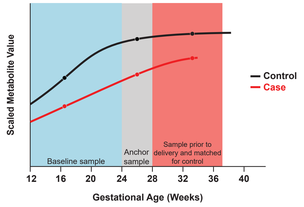


Figure 3: Longitudinal metabolite curves proposed for the larger study

Table 2: Characteristics and ART exposure at time of infant sampling

	Preterm		Term	
Treatment	ZDV	PI-ART	ZDV	PI-ART
N	13	24	11	23
Male Gender (%)	4 (31)	11 (46)	3 (28)	14 (61)
Birth Weight (grams)	2696	2437	2865	2857
GA Delivery (weeks)	32.3	33.1	41.0	39.8

SUMMARY

- Metabolomics is a powerful method to identify metabolic pathways perturbed by ART
- We identified a novel metabolite, 17α - hydroxypregnanolone glucoronide, that is associated with preterm birth regardless of ART regimen
- We identified metabolites associated with preterm birth that were specific to ART regimen
  - 5 α-pregnan-3β-ol,20-one sulfate in PI-ART
  - methionine sulfone in ZDV
- We also identified metabolites known to be associated with preterm birth in untreated women (e.g. urate)
- Infant samples shortly after birth also appear to give insight into metabolites altered by ART that have been previously associated with preterm birth
  - dehydroepiandrosterone sulfate (DHEA-S)
- Given that PTB can be spontaneous, HIV-associated and ART-associated, **large sample sizes** will be needed to tease out the different pathways involved

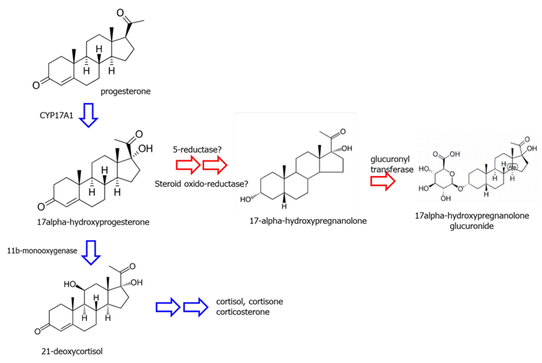


Figure 2: Potential pathway that could lead to the production of the novel metabolite

FUTURE DIRECTIONS

- We are investigating 17α-hydroxypregnanolone glucuronide as a marker of preterm birth (Fig. 2)
- We have submitted a grant proposal to investigate PTB and small for gestational age performing metabolomics on a large number of samples
  - Includes longitudinal sampling and the generation of metabolite curves (Fig. 3)
  - Includes plasma anchor sample so future metabolomic studies of newer ART regimens can be directly compared
  - Creates untreated metabolite curves