Differential DNA methylation between HEU and HUU infants from South Africa

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SOUTH AFRICA, HIV, AND THE BURGEONING HEU POPULATION

- Prevention of mother to child transmission has been successful, but growing evidence suggests **HIV-exposed uninfected** (**HEU**) infants are not completely free of adverse health effects when compared to **HIV-unexposed uninfected** (**HUU**) infants
  - Immune response to vaccines altered
  - Growth delay
  - Neurodevelopmental delay
- ~22% of South African infants are born HEU
  - ~25% HIV prevalence amongst women between 15 and 49 years
- HEU populations are growing worldwide
- DNA methylation (DNAm) is an epigenetic modification which can change in response to environmental stimuli
  - It represents a useful marker for identifying the effects of maternal environment on the developing foetus

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METHODS

Study Participants & Biological Sampling

- Drakenstein Child Health Study
- Mothers recruited, infants enrolled at birth
  - All women diagnosed as HIV-positive prior to or during pregnancy were on antiretroviral therapy
- Umbilical cord blood collected at birth
  - DNA methylation alterations from foetal tissue (umbilical cord blood) may represent infant **responses to the altered maternal environment**
  - Methylation and genotyping data collected
    - Infinium Human Methylation EPIC BeadChip
    - Infinium Human Methylation 450K BeadChip
    - Infinium PsychArray BeadChip
    - Infinium Global Screening Array BeadChip

DNA Methylation Analysis

- Pre-processing to remove “bad” probes, “bad” samples
  - Probe detection p-value > $1x10^{-16}$; cross-hybridising probes; mismatching predicted and reported sex
- Cell type prediction, epigenetic gestational age prediction
  - Epigenetic gestational age acceleration
- All models included sex, principal components of cell type, and principal components of genetic ancestry to account for factors known to affect DNA methylation
- ANCOVA models used to assess:
  - DNA methylation differences between HEU and HUU infants
  - DNA methylation differences between HEU infants exposed to different ARV regimen types

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DNA METHYLATION IS ALTERED AT 14 DISTINCT LOCI BETWEEN HEU AND HUU INFANTS

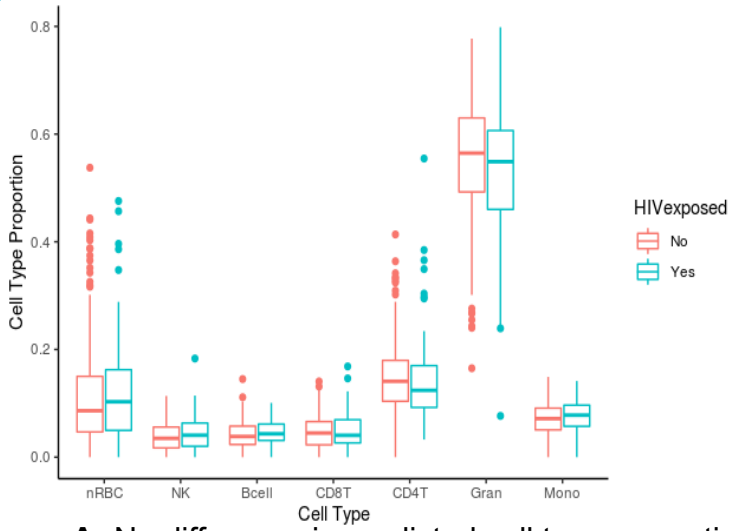

Figure A: No difference in predicted cell type proportion between HEU and HUU infants

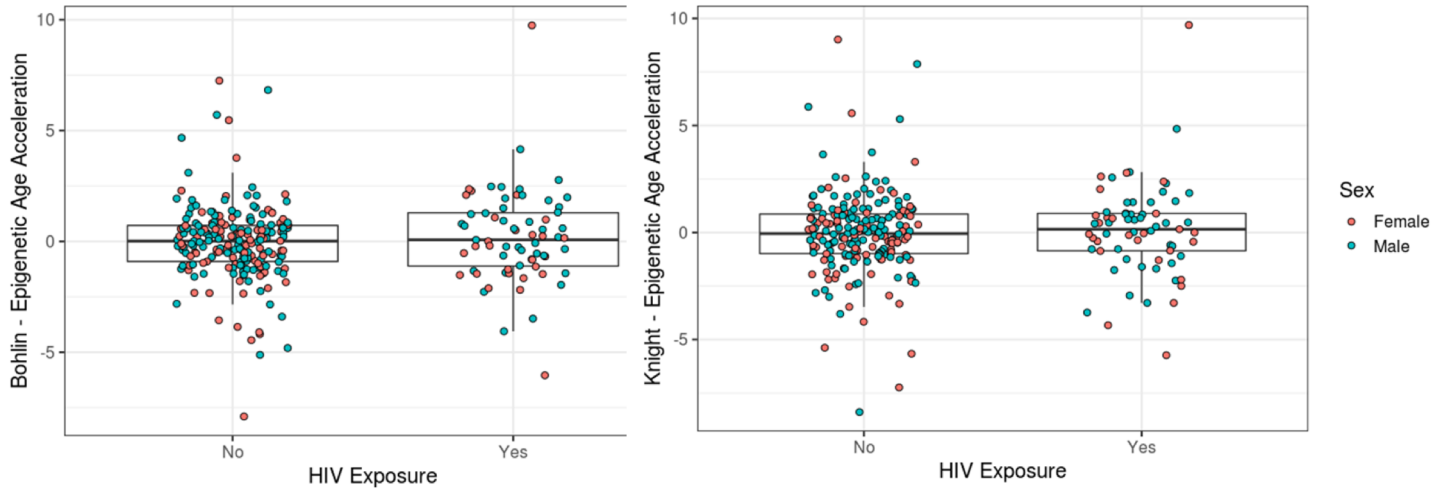

Figure B: Epigenetic gestational age predictions do not differ between HEU and HUU infants according to both the Knight and Bohlin predictors [7,8]

Table 1: Infant demographics do not indicate any significant differences between HEU and HUU infants at birth

	HEU	HUU
N	66	202
Female (%)	29 (44%)	90 (44%)
Birth weight (kilograms)	3.13	3.08
Gestational age (weeks)	38.76	38.82
Ethnicity	59 Black 7 Mixed Race	90 Black 112 Mixed Race
Any maternal tobacco use during pregnancy	10 (15%)	66 (33%)

Table 2: Chromosomal context of EWAS CpG loci (delta beta > 5%, false discovery rate < 5%)

CpG	Chr	Gene	Genomic Context	Epigenomic Context	Delta Beta	p-value	FDR
cg04750100	2	*LCT*	TSS1500		0.054	8.8E-06	0.019
cg19075225	2	*SNED1*	Body		0.061	1.8E-05	0.023
cg10288030	3	*UTS2D*	3'UTR		0.056	1.0E-04	0.049
cg21811021	4			Island	0.053	1.0E-04	0.048
cg22123784	8				0.057	9.2E-07	0.006
cg18419977	11	*SLC22A18*	TSS1500	North Shelf	0.052	4.4E-05	0.035
cg21970626	13			Island	0.068	1.9E-05	0.024
cg04193835	16			Island	0.054	1.4E-07	0.003
cg03238702	17				0.085	1.5E-08	0.001
cg08587775	19			Island	0.064	6.9E-05	0.041
cg17434634	19			Island	0.051	2.5E-05	0.027
cg17759252	19	*CRTC1*	Body	Island	0.065	8.1E-05	0.044
cg14586373	22	*KCTD17*	TSS1500	North Shore	0.057	9.6E-05	0.048
cg21401457	22			Island	0.051	5.5E-06	0.015
cg11747499*	12	*SSH1*	TSS1500	South Shore	0.009 (type 2 v type 1 regimens)	6.1E-07	0.036

Type 1: NRTI + NRTI + NNRTI (n = 51)
Type 2: NRTI (n = 12)
Type 3: NRTI + NRTI + PI (n = 3)

*indicates the sole EWAS hit from investigating DNAm differences between HEU infants exposed to different types of ARV regimen

CONCLUSIONS

- At birth, cell type proportions and epigenetic gestational age do not differ between HEU and HUU groups (Table 1, Figure A, Figure B)
  - There is no obvious immunological challenge at birth, nor are there differences in clinical gestational age in this cohort
- **HIV exposure *in utero*** (and concurrent ARV exposure) **is associated with 14 differentially methylated CpG loci** (Table 2)
  - These loci do not overlap with those identified in HEU infants from the east coast of South Africa
  - The loci are situated in genes which may have roles in metabolism
  - The implications of ARV exposure should be monitored more closely as HEU children age
- One CpG locus, distinct from those related to HIV exposure, is differentially methylated when comparing 2x NRTI + NNRTI and NRTI-based antiretroviral therapy exposure

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