Altered DNA Methylation of *CCR5* in Sigmoid Mucosal Tissues of Transgender Thai Women

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Background

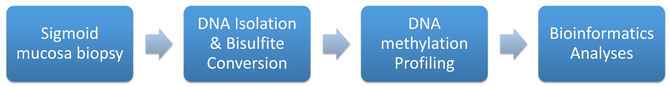
- Transgender women (TGW) have a high global prevalence of HIV infection[1].
- The increased prevalence of HIV infection in TGW has been associated with behavioral and socioeconomic factors[2]. Yet, TGW-specific data examining biological factors linked to HIV acquisition has not been well studied.
- Hormone use has been shown to alter epigenetic patterns and transcriptional regulation in human tissues[3]. Yet, few epigenetic studies have evaluated DNA methylation in tissues and TGW.
- Epigenetic studies of TGW may increase knowledge to better understand the link between exogenous HIV acquisition risk factors and altered HIV-related cell gene regulation in tissues leading to increased risk of HIV transmission.

Methods

- We investigated cross sectional DNA methylation differences in sigmoid mucosa biopsies from cisgender women not on hormonal contraception (CW, n=9), men who have sex with men (MSM, n=10), and TGW who had undergone gender affirmation surgery and remained on hormonal therapy (n=3) in the RV304/SEARCH013 cohort. All study participants were confirmed to be HIV uninfected.
- DNA methylation was measured genome-wide using the Illumina HumanMethylationEPIC array.
- Differential methylation analysis utilized linear models employed in the limma R package and were corrected by false discovery rate.
- Immunophenotyping of peripheral and mucosal mononuclear cells was performed to assess T cell subsets for CCR5 expression by flow cytometry.

Hypothesis

- Exogenous factors such as hormone use alter HIV-related target gene regulation in tissues leading to increased risk of HIV transmission in TGW.



Clinical Results

- Among TGW, mean age of hormone initiation 14yrs, and sex reassignment surgery 19yrs.
- Median duration of hormone use in TGW was 8.5yrs, with 1/3 using estrogen/ progesterone, and 2/3 estrogen only.
- All TGW reported anal and neovaginal sex and median lifetime sexual partners was significantly higher in TGW compared to CW (P = 0.005).

References

1.Baral, Stefan D., Tonia Poteat, Susanne Strömdahl, Andrea L. Wirtz, Thomas E. Guadamuz, and Chris Beyrer. 2013. "Worldwide Burden of HIV in Transgender Women: A Systematic Review and Meta-Analysis." The Lancet Infectious Diseases 13 (3): 214–22.
2. Johansson, Annelie, Domenico Palli, Giovanna Masala, Sara Grioni, Claudia Agnoli, Rosario Tumino, Maria Concetta Giurdanella, et al. 2019. "Epigenome-Wide Association Study for Lifetime Estrogen Exposure Identifies an Epigenetic Signature Associated with Breast Cancer Risk." Clinical Epigenetics 11 (1): 66.
3. Poteat, Tonia, Mannat Malik, Ayden Scheim, and Ayana Elliott. 2017. "HIV Prevention Among Transgender Populations: Knowledge Gaps and Evidence for Action." Current HIV/AIDS Reports 14 (4): 141–52.

Results

1. Genome-wide differences in epigenetic mark DNA methylation associated with TGW.

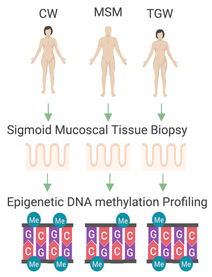


Figure 1: Diagram of study design (left).

Comparison	# DNA Methylation sites differentially methylated at *P*<0.05 FDR Corrected
MSM vs. CW	5,162
MSM vs. TGW	219,788
CW vs. TGW	188,833

2. TGW-related DNA methylation changes occur at 5' UTR of regulatory region of *CCR5* gene.

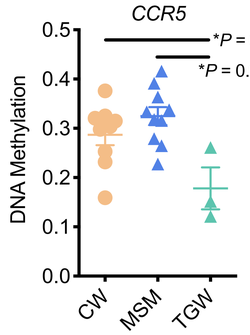


Figure 2: Significant DNA methylation changes in gut mucosal biopsies at CpG related to *CCR5* gene (One-way ANOVA). CW participants displayed as orange, MSM displayed as blue, and TGW displayed as green.

3. Mucosal CCR5+ CD4 T cell frequencies significantly higher in TGW compared to MSM.

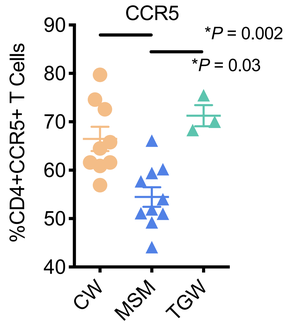


Figure 3: Significant changes in CCR5+ CD4 T cells in gut mucosal biopsies (One-way ANOVA). CW and TGW significantly higher compared to MSM. CW participants displayed as orange, MSM displayed as blue, and TGW displayed as green.c

4. DNA methylation changes occur at 5' UTR of regulatory region of *CCR5* gene associate with mucosal CCR5+ CD4 T cell frequencies

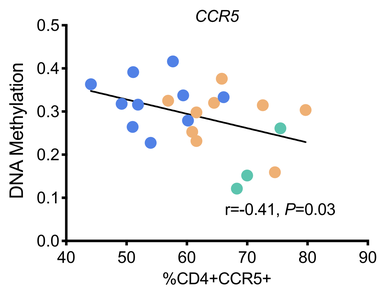


Figure 4: Significant association of DNA methylation levels at *CCR5* gene and %CD4+CCR5+ cells in gut mucosal biopsies. CW participants displayed as orange, MSM displayed as blue, and TGW displayed as green.

Conclusions

- The gut mucosal epigenome appears to be altered in TGW compared to CW and MSM Thai volunteers.
- There were fewer differences between TGW and CW (188,833 methylation sites) and even fewer between CW and MSM (5,162 methylation sites), suggesting a unique gut mucosal compartment may exist in TGW.
- Of known HIV acquisition risk genes, methylation at CpGs at the promoter region of the CCR5 gene were significantly decreased in TGW compared to both CW ($P < 0.05$) and MSM ($P < 0.05$).

Future Directions

Further investigations of biological HIV risk factors specific to TGW are needed to inform additional HIV prevention strategies.

Acknowledgements

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Disclaimers

The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.