Analyzing mtDNA in Plasma-Derived Extracellular Vesicles from Pregnant Black Women to Predict Adverse Outcomes

Iman Dorman, COL 2026, PURM, Kobe Abney, B.S., Thea Golden, PharmD/Ph.D., Yu-Chin Lien, Ph.D., Nadav Schwartz, M.D., Rebecca Simmons, M.D.
Center for Research on Women's Health and Reproductive Medicine, University of Pennsylvania Perelman School of Medicine

Abstract

In the US, Black women experience two to three times higher rates of adverse pregnancy outcomes and pregnancy-related mortality compared to their White counterparts.[1] This disparity persists despite adjustments for socio-economic factors like education and income, showcasing the impact of structural racism and discrimination. These factors may have biological implications, leading to an elevated risk of adverse pregnancy outcomes driven by placental dysfunction. Placental dysfunction occurs when the placenta fails to deliver sufficient oxygen and nutrients to the fetus, linked to outcomes such as preeclampsia and preterm birth.[2] Our lab previously identified significant placental differences in metabolic and transcriptional factors of healthy Black women versus White women, indicating reduced resilience to additional stress. The current study uses extracellular vesicles (EVs) to understand metabolic and placental differences between first-trimester Black women with adverse vs non-adverse outcomes. EVs are a heterogeneous population ranging from small (30-200 nm) to large (200-1000 nm) that are released into the extracellular environment by all cell types. EVs transport RNAs, proteins, and mitochondrial components between maternal and placental cells, influencing cellular functions in recipient cells and reflecting the pathological states of their cells of origin. This study assesses the potential of circulating plasma-derived EV from first-trimester Black women as early indicators of adverse outcomes. Using the mtDNA content of EVs as a marker of cellular stress, we hypothesize that small EVs from women with adverse outcomes will exhibit elevated mtDNA levels, indicative of increased stress.

Clinical Demographics

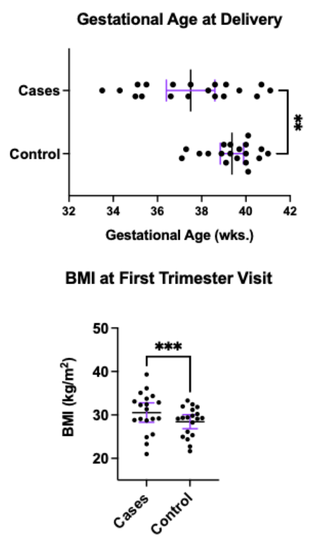

Fig 1. Case-control cohort of n=19 women diagnosed with pre-eclampsia, preterm birth, gestational hypertension, or fetal growth restriction were initially matched on race, maternal age, BMI, and health insurance status.[3] These matches were not used in the current analysis due to unreliable mtDNA measurements in some samples.

Methods

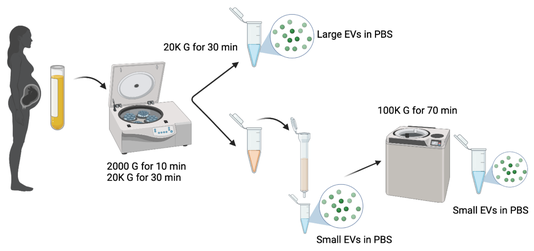

Figure 2. small EVs are extracted from Black women's plasma using a two-step process: differential centrifugation followed by Izon qEV size exclusion chromatography.

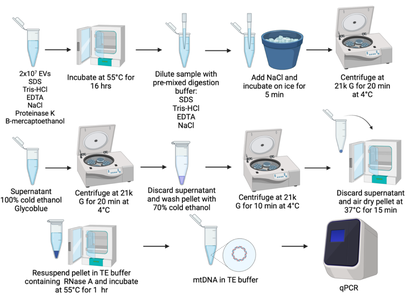

Figure 3. mtDNA is extracted from small EVs. The mtDNA copy number is quantified using qPCR, following the protocol established by Kaufman et al. [4]

Results

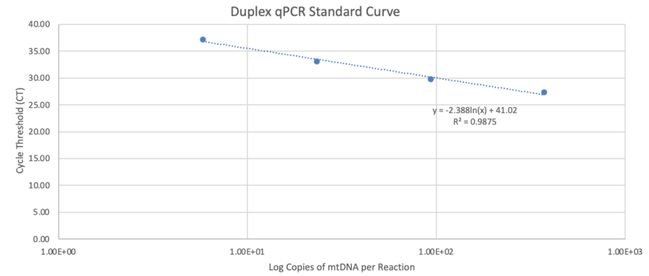

Figure 4. mtDNA content per EV was quantified by aligning sample CT values to a standard curve and normalizing to total EV count.

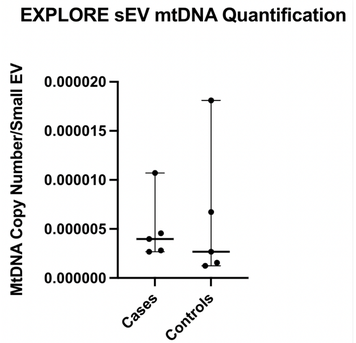

Figure 5. Plotted is the median copy number per EV with 95% confidence intervals. A Mann-Whitney test was performed.

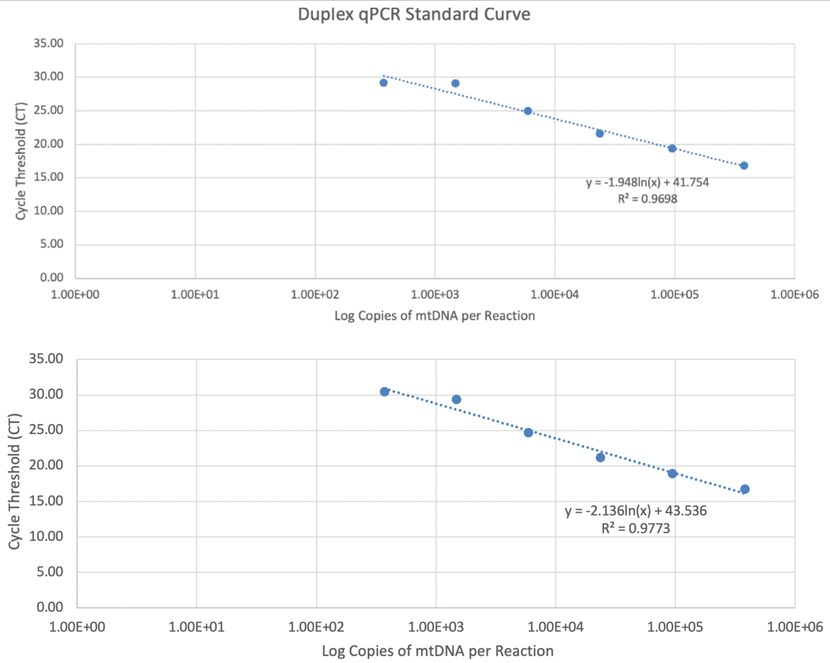

Figure 6. Standard curves from pre-experimental qPCR runs focused on improving the technical skills required for the study's quantification method and to measure efficiency of primers used in qPCR.

Conclusion

- No statistically significant difference between groups but a visible trend of higher copy numbers in cases compared to controls.
- There is a potential biological difference that may be significant with an increased sample size.

Future Directions

- Assess the quality mtDNA in EVs using long amplicon quantitative which quantifies the degree of polymerase-inhibiting lesions present due to single-strand breaks and bulky adducts. [5]
- Correlate the release of undamaged mtDNA from cells into EVs with triggering an inflammatory response, versus damaged mtDNA, which may contribute to significant cellular pathology.

References/ Acknowledgements

[1] Hill et al., (2022). Racial Disparities in Maternal and Infant Health: Current Status and Efforts to Address Them. Retrieved from Kaiser Family Foundation.
[2] Zhang et al., (2020). PMID: 32175696
[3] Unpublished work form Pharmacology Graduate Student Kobe Abney
[4] Kaufman et al., (2020). PMID: 32900851
[5] Gonzalez-Hunt et al., (2016). PMID: 26828332

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