

Variability of Salivary microRNA Expressions: Preliminary Implications for Concussion Biomarkers in Military Populations

Nicholas Reilly¹, Haley Chizuk², Maria Gonnella¹, Mackenzie Ferguson³, Jessica Roberson¹, Alyssa Lickfeld², Anne Grello³, Steven Hicks⁴, Jeffrey Miecznikowski⁵, Mohammad Haider², John Leddy²

1. Geneva Foundation, Fort Bragg, NC, 2. UBMD Department of Orthopaedics and Sports Medicine, Jacobs School of Medicine and Biomedical Sciences, SUNY University at Buffalo, Buffalo, NY, 3. Geneva Foundation, Camp Lejeune, NC, 4. Department of Pediatrics, Penn State College of Medicine, Penn State University, Hershey, PA, 5. Department of Biostatistics, School of Public Health and Health Professions, SUNY University at Buffalo, Buffalo, NY

Background

- Concussion is the most common form of traumatic brain injury (TBI) observed in the Department of War (DoW) with over 450,000 diagnosed injuries since the start of the 21st Century.
- Improvements in diagnostic and screening procedures for concussion could have significant impacts on acute and long-term outcomes following concussion in service members.
- Salivary microRNAs (miRNAs) have emerged as relevant, rapid, and non-invasive fluid biomarkers for numerous DoW health concerns, including concussion.
- However, assessments of miRNAs as biomarkers for concussion within military populations have been, to date, limited in scope.

The primary objective of this study was to assess the temporal stability of concussion-relevant miRNA panels within a non-injured active-duty population to:

(1) establish miRNA as a stable physiological marker over time and (2) ensure that observed differences reflect injury-related changes rather than background biological variability.

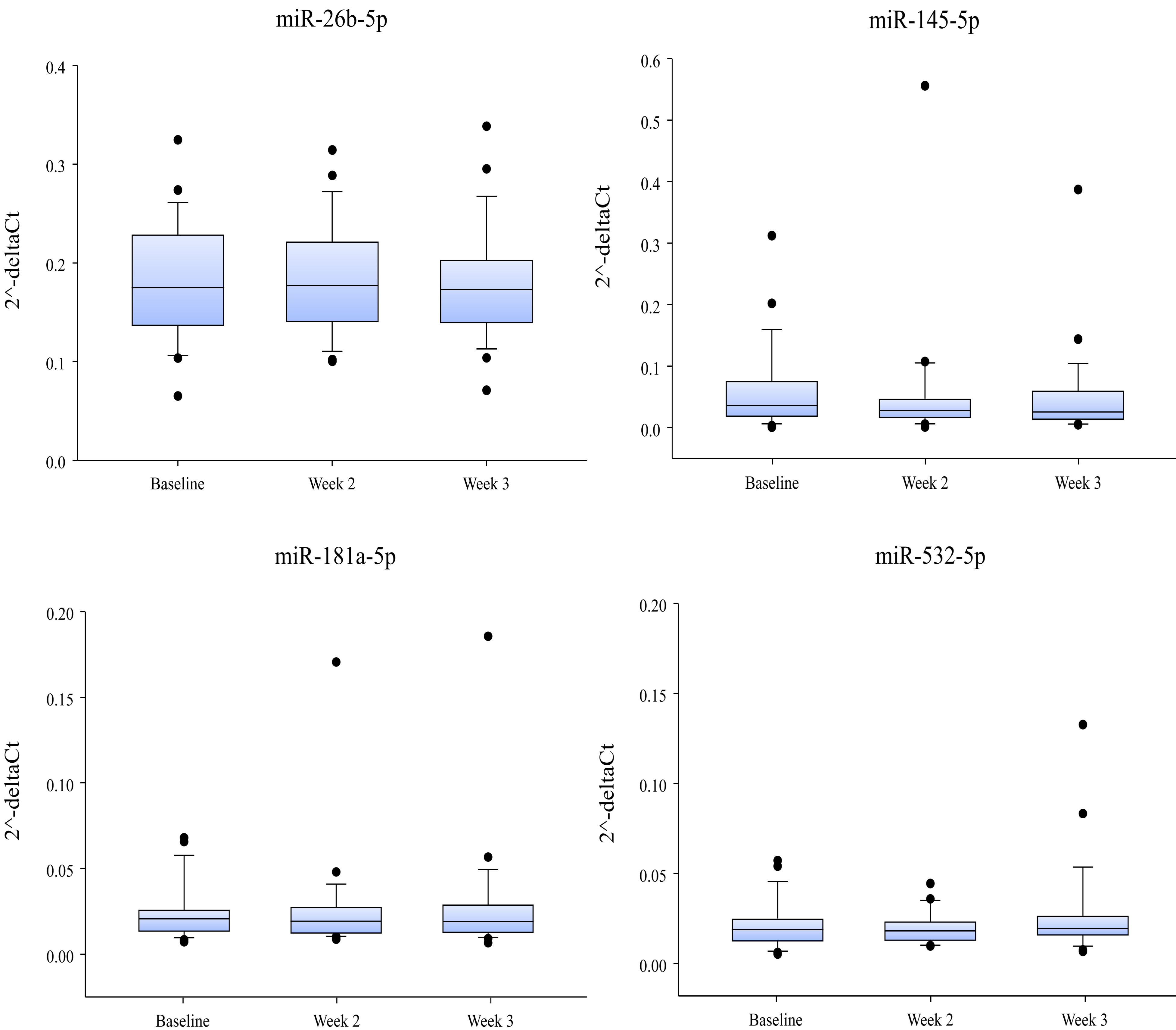
Methods

- Following IRB approval and informed consent, 25 active-duty service members (ADSMs; aged 26.2 ± 5.18 ; 92 % male) were recruited to participate as part of a larger randomized controlled trial.
- Target miRNAs selected for analysis were based on their established relevance to concussion and included miR-26b-5p, 181a-5p, 141-5p, and 532-5p, with miR-16-5p serving as the endogenous control.
- Participants provided weekly salivary samples over the course of three weeks. Saliva samples were assessed in triplicate using quantitative real-time polymerase chain reaction (qRT-PCR) to determine miRNA expression.
- Kruskal-Wallis tests were conducted to assess inter-individual and miRNA stability over time, respectively.



ORAcollect-RNA ORE-100.025 swab collection kit (DNA Genotek, Ottawa, Ontario, Canada)

Results



Sample Quality

- 97.6 percent of samples collected across all timepoints showed adequate amplification of the control gene, miR-16-5p, and were utilized for statistical analyses.

Repeated-Measures Stability

- No significant differences observed for all miRNA expressions at baseline ($p > 0.05$).
- No significant intra-individual differences in miRNA expressions were observed across all timepoints for miR-26b-5p ($p=0.839$), miR-145-5p ($p=0.986$), miR-181a-5p ($p=0.899$), and miR-532-5p ($p=0.802$), respectively.

- Stable expressions across multiple time points indicate potential utility for these miRNA strains as indicators of sustained neurotrauma and/or the lack thereof in healthy individuals.

Inter-Individual Stability

- Upon visual inspection, all target miRNAs showed consistent inter-subject distribution patterns.
- Consistent inter-individual expressions are critical for future identification of outliers, normative values, and intra-individual references for determining a sustained injury.

Conclusions

The target miRNAs analyzed in this study were shown to be stable across multiple timepoints within non-injured ADSMs, supporting their potential utility as physiologically stable biomarkers for concussion research within the military.

- These initial findings provide foundational evidence that observed expression changes in future injury cohorts may reflect true biological responses rather than baseline variability.
- Further discussion on the use of miRNA as an adjunct to clinical screens for concussion within the Military Health System is warranted to possibly supplement diagnostic procedures and improve treatment outcomes.

Acknowledgments

- This work was part of a larger clinical trial (NCT05498038) funded by the Congressionally Directed Medical Research Program (CDMRP; W81XWH-22-1-1115).
- The views expressed here are those of the authors and do not necessarily reflect the official policy or position of the Defense Health Agency, Department of War, nor the United States Government.
- The authors extend their sincerest gratitude toward all study personnel and participants for their contributions toward this work.