Biomarkers of PTSD: Dried Blood Spot mRNA Isolation, Amplification, and Analysis

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Introduction

• Post-traumatic stress disorder (PTSD) results in strong negative changes to mood and behavior after exposure to violent and/or traumatic events. However, diagnosing PTSD is often hindered by the inaccuracy and stigma of self-reporting of symptoms, highlighting the need for a biomolecular test for improving PTSD diagnosis.

• Dried Blood Spots (DBS) hold genetic information in the form of mRNA that may be valuable source material for biomarker analysis when analyzed for differential gene expression.

• We attempt to extracting total RNA from dried blood spots in order to demonstrate viability of using mRNA transcriptomes as a basis for biomarker-aided PTSD diagnosis. Improving this extraction process required designing and modifying protocol from initial blood collection to final genetic analysis (Figure 1 below).

Key Results

• Total RNA from 2.5 drops of whole capillary blood was dried on cellulose paper and extracted via silica spin-column based RNA purification and then visualized using denaturing gel electrophoresis and SYBR™ Gold nucleic acid stain.

• DNA oligomer PCR primers targeting PTSD-linked genes of interest were designed, evaluated in silico, then tested. 14 of these primers successfully amplified genetic material extracted from dried capillary blood samples.

• Produced improved capillary whole blood sampling protocol and graphic instruction materials intended for more reliable home-kit usage.

Conceptual Framework

• This research builds upon previous work using venous blood to and filter paper to preserve nucleic acids for later downstream applications.

• However, detailed methodology for consistent, high-efficiency, low-degradation total RNA prep using silica columns and cellulose paper has been either poorly documented or has proven difficult to reproduce experimentally.

• Our methodology has demonstrated that RNA preservation, isolation, and amplification can be accomplished by users of undergraduate-level experience and that PCR amplification can be used to amplify PTSD-relevant genes and mRNA transcripts using standard molecular biology equipment and reagents and off-the-shelf reaction kits.

Impact/Conclusions

• Our work has demonstrated the potential viability of DBS mRNA extraction for use of detecting PTSD-relevant mRNA transcripts, as well as sufficient quantity and purity for downstream applications.

• Molecular testing for mRNA transcripts demonstrates incredible potential for enhancing the accuracy and availability of psychiatric diagnosis using molecular in vitro testing to augment traditional in-person evaluation.

Acknowledgements

Many thanks go to the following individuals and organizations for their exceptional mentorship and support: UAH Office of the President, Office of the Provost, Office of the Vice President for Research and Economic Development, The Dean of the College of Science, the Dean of the College of Engineering, the Alabama Louis Stokes Alliance for Minority Participation, and the Alabama Space Grant Consortium.

Reference