INTRODUCTION

- Adverse posttraumatic neurological sequelae (APNS) such as posttraumatic stress, depression, and persistent pain are common following traumatic stress exposure.
- Little is known about the underlying biological mechanisms driving APNS development and persistence. Increased understanding of genetic and molecular mechanisms could lead to the development of novel therapeutic strategies to prevent and treat APNS.
- In addition, few risk biomarkers have been identified that predict APNS, limiting the ability of clinicians to accurately identify trauma stress survivors who are at high risk of APNS and in greatest need for intervention.

Aims

- To collect high-quality participant blood and plasma samples from men and women enrolled in the AURORA study at three timepoints following traumatic stress exposure.
- To process participant samples and generate high-quality blood-based analyte and -omic data (genetic, epigenetic, transcriptomic, miRNAic).
- To leverage this data to identify promising risk biomarkers and to elucidate molecular mechanisms of APNS development/persistence.

METHODS

Sample collection – Blood was collected from participants into DNA PAxGene, RNA PAxGene, and EDTA tubes in the Emergency Department (ED), and at 2 weeks and 6 months following traumatic stress exposure. Venipuncture was performed by research assistants in the ED. For the follow-up timepoints, participants either returned to the ED, had blood collected while following up for neuroimaging visits, or had blood collected via mobile Heathby. EDTA tubes were processed into plasma within 30 minutes of collection. All tubes were frozen at -20°C until shipping. To ensure high quality sample collection, sample metrics such as blood volume, time to process, time to freeze, and plasma color/fairness were continuously assessed and improved throughout the study and are documented in separate protocol.

Sample shipping and storage – All samples were shipped in batches on dry ice from study sites to the NIH Repository & Genomics Resource (NRRG). DNA was isolated from DNA PAxGene tubes upon arrival to the repository using magnetic bead technology via Chemagic 360 instrument. Samples were immediately processed on a SNP array (Fluidigm) to confirm sample identity and quality. Sample processing – DNA genotyping was performed on all participants using the Global Screening Array (Illumina). All other blood-based biological sample processing, including Cpg methylation (EPIC array, Illumina), total RNA sequencing, small RNA sequencing, and plasma protein/hormone quantification (LC-MS ELISA, Mesoscale) was performed on a subset of AURORA participants, with prioritization of participants whose blood was collected at all three timepoints.

DNA sample processing- Genotyping: A total of 3,684 samples were genotyped at the Stanford Center for Invariant using the Infinium Global Screening Array-24 v1.0 (Illumina Inc., San Diego, CA) across five batches between June 2019 and November 2021. A total of 688,032 variants were queried on the array. Genetic data was cleaned and subsequently imputed to generate over 13 million total SNPs. Genetic data was used to estimate genetic sex and genetic ancestry and to calculate polygenic risk scores.

RNA sample processing- RNA was isolated from PAxGene/RNA tubes using PAxGene blood RNA kits (Qiagen) and RNA integrity was assessed using TapeStation (Agilent). Total RNA: Libraries were constructed using TECAN genomics UniversalPlus Total RNA Seq kits with RNA and globin depletion. Sequencing was performed on NovaSeq 5 S4 (Illumina) using Paired-end, 100bp reads, achieving >100 million reads per sample. RNA seq reads were aligned to protein coding regions of the genome, normalized, transformed, and outliers removed. Over 16,000 gene transcripts were detected above a threshold of five sequencing reads per sample. microRNA Libraries were constructed using NexFlex small RNA seq kits v4 (Perkin Elmer/lumina), sequencing on NovaSeq S4 with Single-end 50bp reads. RNA seq reads were aligned to mature miRNA sequences, normalized, transformed, and outliers removed. Over 700 mature miRNA were detected.

Plasma processing- Plasma was isolated from whole blood via centrifugation and aliquoted into 12-250ul aliquots. Aliquots were used to measure multiple analytes. 17β-estradiol was measured via ELISA. Additional circulating hormones and proteins were measured via liquid chromatography tandem mass spectrometry (LC-MS). Briefly, the organic layer was extracted using methanol-butyl ether, dried, and reconstituted in methanol. Analyses were performed with a ThermoFisher TSQ Vantage coupled to a Waters Acquity UPLC. Cytokines were measured via MesoscaleDiscovery following extensive technical validation testing of multiple analysis platforms.

RESULTS

Blood and blood/plasma were successfully collected from AURORA study participants at three timepoints following traumatic stress exposure: ED, 2 weeks, and 6 months. Samples were shipped to NRRG, where they are currently stored. A subset of these samples were checked out of the repository and used for data processing as indicated in Boxes A (DNA), B (RNA), and C (plasma).

Genetic, methylation, total RNA sequencing, small RNA sequencing, and protein/hormone data from blood/plasma are available for a subset of the AURORA participants and timepoints as indicated in each box. For data types with longitudinal samples (i.e., all data types except for SNP-based genotyping), a further subset of participants have data from multiple timepoints. For example, n=90 participants have Cpg methylation data for all three timepoints, n=177 have paired Cpg methylation data for ED and 2 weeks timepoints, and n=177 participants have paired Cpg methylation data for ED and 6-month timepoints.

Limited data analyses were presented. However, data presented, e.g. polygenic risk scores, differential gene expression analyses, and assessment of the relationship between peritraumatic 17β-estradiol and persistent pain outcomes, represents just a small sampling of potential studies that could be performed with this data.

CONCLUSIONS

• Sample collection is complete, sample processing is underway (mostly complete), and data analyses are ongoing. Preliminary quality assessment data indicate that data collection and processing methods are of high quality (e.g. blood collected from >95% of all AURORA participants, technical validation of cytokine level data, etc.).

• All processed data will be publicly available via the NIH Data Archive. For questions or comments related to this data, please email sarah_linstaetd@med.uc.edu.

REFERENCES


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