**INTRODUCTION**

- Chronic posttraumatic musculoskeletal pain (CPMP) is a common outcome of traumatic stress exposure and is highly morbidity and costly (1).
- Women are disproportionately burdened by CPMP (3,4).
- Few biomarkers have been identified (3).

In a previous study, we showed across three independent longitudinal cohorts of trauma survivors that peritraumatic circulating levels of 17β-estradiol (E2) are a strong predictor of CPMP in women (but not men), such that higher circulating levels of E2 in the early aftermath of trauma exposure was associated with clinically and statistically significant lower levels of CPMP over the course of a year following trauma (5).

In the current study, we aimed to further validate these findings using samples and data from the AURORA study (6). Further, we aimed to assess potential mechanisms in which E2 influences CPMP using available miRNA data from the same human cohort and complimentary in vitro cell-based studies.

**HYPOTHESIS**

Higher levels of E2 in the early aftermath of traumatic stress exposure is associated with lower levels of CPMP. miRNA mediated the relationship between E2 and CPMP development, and these miRNA were involved in neuronal processes important to the pathogenesis of CPMP.

**METHODS**

The AURORA study is a longitudinal cohort designed to improve the prevention, diagnosis, and treatment of adverse posttraumatic neurophysiological sequelae, including CPMP, following traumatic stress exposure. Participants aged 18-75 years who presented to the ED within 72 hours of trauma exposure at 23 participating ED sites were enrolled. Overall musculoskeletal pain severity was assessed in the immediate aftermath of trauma and at three follow-up timepoints (eight weeks, three months, and six months) via a 0-10 numeric rating scale (NRS: 0 = no pain) to 10 (maximum possible pain). Blood samples were collected in the aftermath of traumatic exposure into EDTA and PaxGeneRNA tubes. 17β-estradiol levels were measured in duplicates using UltraSensitive Estradiol Enzyme Linked Immunosorbent Assay (ALPCO, Salem, NH, Catalog #20-ESTHUE-01) according to manufacturer’s specifications. Sample absorbance (450nm) was measured using a Synergy HTX Multi-mode Microplate Reader (BioTek Instruments). Hormone concentrations were calculated from a standard curve generated using samples with known concentration; these standards were included on each 96-well plate containing study samples. We also measured known concentrations and two samples in duplicate that was measured across all plates to control for cross-plate variability. For RNA analysis, template libraries for miRNA next-generation sequencing were produced from 1.0 ug total RNA. Samples were prepared using PreqA Biolexic miRNA library prep kits according to manufacturer’s instructions (Qiagen, Germantown, MD). RNA sequence reads were processed using a custom bioinformatics pipeline and were normalized using upper quartile normalization. We examined the direct transcriptional impact of 17β-estradiol on dorsal root ganglia (DRG), known centers for nociception-related gene regulation by estrogen receptor signaling (7). Lumber DRG (L4-L6) were dissected and prepared from naïve, female Sprague-Dawley rats (4-5 weeks old) and prepared cells were stimulated with 100-nM 17β-estradiol (Sigma, E2758). RNA was isolated after 3 hours using TRIzol Reagent (Invitrogen, Carlsbad, CA, 155602B) and miRNA expression changes were measured using the GeneChip™ miRNA 4.0 Array (Thermo Fisher Scientific, CA, 92411). Statistical Analyses. E2 levels were determined to be non-normally distributed via the Shapiro-Wilk test; therefore, E2 levels were transformed by taking the square root of raw E2 values. Linear mixed models were used to assess the relationship between peritraumatic E2 levels and CPMP using the linear function from the ime4 package (v. 1.1.238) (8) R v. 3.2.2 (9) and adjusted for age, sex and race. Differential expression statistics were calculated using Linear Models for Microarray Data (LIMMA) and the eBayes (an empirical Bayes moderated-t-statistic) method while controlling for false discovery rate (FDR). A p-value threshold of 0.05 was used.

**RESULTS**

- Characteristics of the study sample are shown in Table 1. Most participants were less than 40 years old, had experienced motor vehicle collision, had some college education, and were overweight or obese.
- In women only, an inverse relationship between peritraumatic E2 and the development of CPMP was observed (β = 0.137, p = 0.033) such that with high E2 at the time of trauma had less CPMP over the following weeks and months.
- Secondary analyses identified 25 miRNA that mediated the relationship between E2 and CPMP: pathway analysis of the 25 miRNA identified "Neurological Disease, Organismal Injury, and Psychological Disorders" as the top network.
- Female rat lumbar DRG treated with E2 had decreased expression of miR-454 and miR-3347. In AURORA women, these miRNA were also downregulated in women with high E2.

**CONCLUSIONS**

We replicated the results from previous cohorts showing that increased peritraumatic E2 levels predict improved CPMP outcomes in women. This relationship might be mediated by E2 regulation of pain-associated miRNA transcripts. Further studies are needed to validate this finding.

**REFERENCES**

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**TABLE 1. Cohort characteristics.** Women (n = 164) and men (n = 187) from the AURORA cohort included. All participants were enrolled in the immediate aftermath of trauma.

**TABLE 2. Repeated Measures Regression Analyses.** The relationship between circulating 17β-estradiol levels at the time of trauma exposure and chronic posttraumatic pain severity over the course of six months (WK1, WK6, and WK11). Table 2A shows the relationship between current and previous CPMP pain severity with associated 95% confidence intervals.

**FIGURE 2. Women with high circulating levels of 17ß-estradiol at the time of trauma exposure were 0.137 lower in pain severity in the subsequent weeks and months (n=164).**

**FIGURE 3. Secondary analyses assessing potential miRNA that mediate the relationship between E2 and chronic pain severity identified 25 full mediators.

**FIGURE 4. Pathways analyses using the 25 miRNA mediators shown in Figure 3 identified Neurological Disease, Organismal Injury, and Psychological Disorders as the top network shared by the miRNA.**

**FIGURE 5. Of the miRNA identified as mediators of the relationship between E2 and CPMP in human AURORA participants, both miR-454 (p = 0.0193) and miR-27b (p = 0.0279) were significantly downregulated in female rat DRG following treatment with E2. This was the same direction of effect as seen in AURORA women.**

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**Supplementary figure:** Nociceptive-Related miRNA**, shown in blue, were significantly downregulated in female rat DRG following treatment with E2 compared to control. The relationship between miRNA expression and pain severity was assessed using functional pathway analysis. The pathway with the most statistically significant enrichment was identified as Neurological Disease, Organismal Injury, and Psychological Disorders (CPC: 0.4375, log 10 p-value: 1.12, FDR < 0.001) and is shown in red.