Expression levels of XIST RNA predict PTSS and chronic pain outcomes in women experiencing motor vehicle collision

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INTRODUCTION
Women experiencing motor vehicle collision (MVC) are at substantially increased risk of both chronic post-traumatic pain (CPTP) and posttraumatic stress symptom (PTSS) severity.

X chromosome inactivation (XCI) is one candidate mechanism contributing to sexual dimorphism in individuals with CPTP and PTSS. The long non-coding RNA, X-inactive specific transcript (XIST), is known to be a major regulator of XCI.1,2 Altered ability of XIST to coat the X chromosome is one factor that facilitates gene escape from XCI; sixty-two human X chromosome genes are known escapes.2,3

Previous studies have shown that 1) XIST RNA is over-expressed in females with major affective disorders,4 and 2) escapee genes are associated with depression, bipolar disorder, and mental impairment4,6.

HYPOTHESIS
Expression levels of XIST RNA predict CPTP and PTSS outcomes in women experiencing MVC, and X chromosome gene transcripts known to escape XCI are correlated with XIST RNA expression levels and these genes are associated with CPTP and PTSS outcomes in women.

METHODS

American women aged (n = 66) age 18 to 65 presenting to one of thirteen Emergency Departments (EDs) after MVC who did not have a fracture or require hospital admission were enrolled. CPTP was assessed at 20 body regions on a 0-10 Numeric Rating Scale (NRS) and PTSS was assessed using the Impact of Event Scale-Revised 6 weeks, 6 months, and 1 year after MVC.

Blood was collected in RNA-PAXgene tubes in the ED and total RNA was isolated using PAXgene blood mRNA kits (PreAnalytiX). Total RNA was purified for sequencing using Ovation Human Blood RNA seq kits (NuCen). Sample libraries were sequenced on a HiSeq2500 system (illumina). Raw sequencing reads were aligned to hg19 using STAR, quantified using RSEM, and normalized to the overall upper quartile.

Repeated measures logistic regression analyses adjusted for age, study site, and time following MVC were used to evaluate the relationship between XIST RNA expression levels and CPTP or PTSS severity. Mean levels of expression in individuals with these outcomes versus those who recovered were derived from marginal means from regression models. Bivariate analyses were used to determine the Pearson correlation coefficients and p values corresponding to the relationship between XIST RNA expression and previously identified escapee genes. Genes that were previously shown to escape XCI were mapped to a schematic of the X chromosome using coordinates derived from hg19 genome build. Competitive gene set analyses4 were used to compare expression levels of genes positively correlated with XIST in the MVC study predict CPTP or PTSS better than any other set of genes, in terms of differential expression. This test accounts for inter-gene correlation. All statistical analyses were conducted using either SPSS (v24) or R statistical programs (camera [limma]).

RESULTS

• American women aged 18 to 65 (Table 1) presenting in the Emergency Department (ED) following MVC were enrolled in the study (n=66). Blood was collected in the ED at the time of enrollment and CPTP and PTSS were assessed (Figure 1). Characteristics of the study population are provided in Table 1.

• Higher XIST RNA expression levels were associated with increased risk of developing CPTP and PTSS six months following MVC (Figure 2).

• Previously defined escapee genes were mapped on the human X chromosome (Figure 3). Most genes that escape XCI are located on the p arm of the X chromosome.2,3 Eighteen of these escape genes (defined in blue, Figure 3) are positively correlated with XIST RNA expression levels (p<0.05) in women following MVC. Example correlations are shown in Figure 4. As a control, we examined the relationship between XIST RNA expression levels and the expression of genes never known to escape XCI, the expression of these transcripts were negatively correlated (data not shown).

• Gene set enrichment analyses demonstrated that the eighteen X chromosome genes correlated with XIST in women who develop CPTP and PTSS following MVC were more differentially expressed than any other set of eighteen genes (Table 2).

• Twelve of the eighteen genes that were positively correlated with XIST were expressed at significantly higher levels in women that developed CPTP and PTSS than women who recovered (p<0.05) following MVC (Figure 5).

CONCLUSIONS
These data suggest that XIST RNA and related X-chromosome transcript levels predict CPTP and PTSS in women experiencing MVC. Further studies are needed to replicate these findings and examine potential mechanisms.

REFERENCES
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*Research reported in this publication was supported by NIMH of the NIH under R01-AR0607852. The Mayday Fund, and an American Pain Society Future Leaders in Pain Grant. The content is solely the responsibility of the authors and does not necessarily represent the official views of these funding agencies.*