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] 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 escapees.[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 impairment[4,5].

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

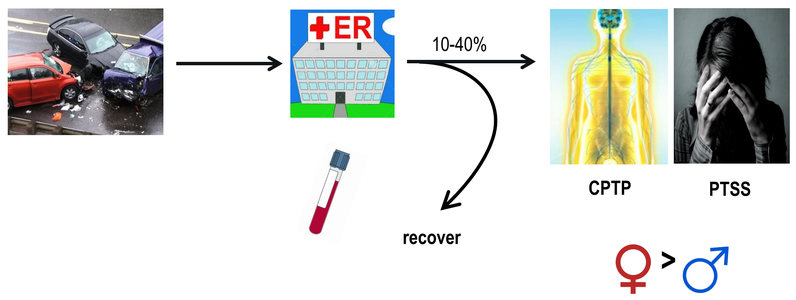
African American women (n = 66) age 18 to 65 presenting to one of thirteen Emergency Departments (EDs) after MVC who did not have a fracture or require hospital admission were enrolled. CPTP was assessed at 20 body regions on a 0-10 Numeric Rating Scale (NRS) and PTSS was assessed using the Impact of Events 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 miRNA kits (PreAnalytix). Total RNA was prepared for sequencing using Ovation Human Blood RNA seq kits (NuGen). 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 recover 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 analyses[6] were used to compare whether 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}).

FIGURE 1 and TABLE 1. Study design and characteristics. Sixty-six African American women were enrolled in one of 13 Emergency Departments in the early aftermath of motor vehicle collision. Blood was collected at the time of enrollment and CPTP and PTSS was assessed 6 weeks, 6 months, and 1 year following MVC.



Characteristic	
Women Enrolled, n	66
Age, years, mean (SD)	30 (10)
Education, n (%)	
HS or less	11 (16.7)
Post-HS not college	3 (4.5)
Some college	35 (53)
College	14 (21.2)
Post-college	3 (4.5)
Collision characteristics	
Driver, n (%)	49 (74.2)
Severe vehicle damage, n (%)	38 (57.6)
Seatbelt worn, n (%)	61 (92.4)
Airbag deployed, n (%)	24 (36.4)
Distress* in the early aftermath of trauma, mean (SD)	25 (11)
Overall pain in the ED (0-10 NRS), mean (SD)	6.9 (2.3)
IESR score at 6 months, mean (SD)	29.9 (28.6)
Overall pain at 6 months, mean (SD)	5.2 (3.4)

*distress measured with the peritraumatic distress inventory

FIGURE 2. *XIST* RNA expression levels are higher in women who develop CPTP and PTSS six months following MVC than in women who recover.

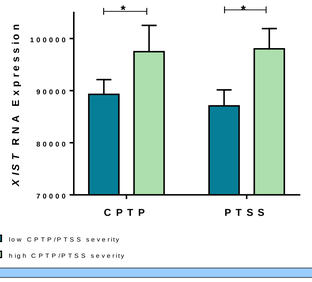


FIGURE 4. Of known escapee genes, eighteen are positively correlated with *XIST* in MVC study participants (example correlations shown below), suggesting potential escape from XCI.

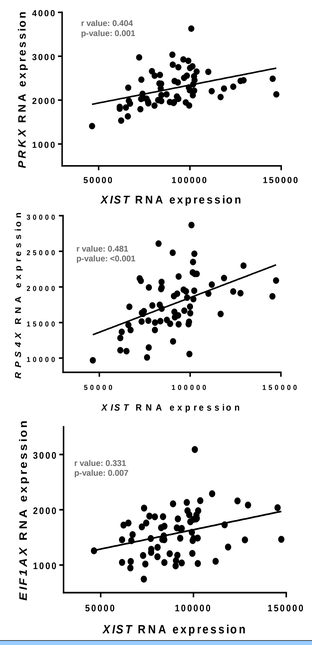


FIGURE 3. X Chromosome genes known to escape XCI. The eighteen genes that are positively correlated with *XIST* in women following MVC are shown in blue.

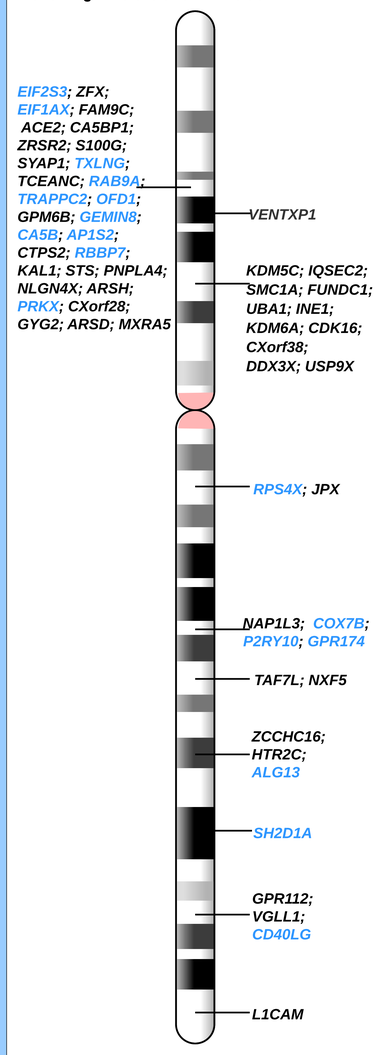


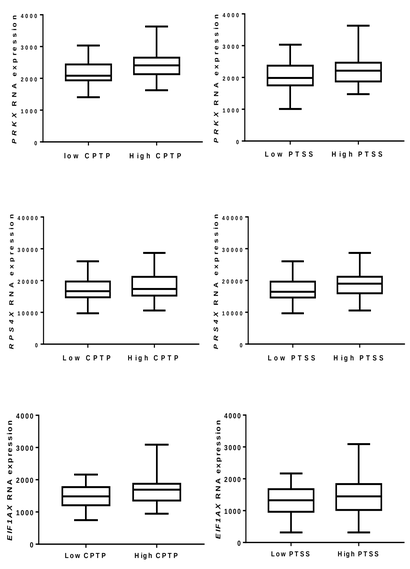
TABLE 2. Competitive gene set analyses show that the set of eighteen X chromosome genes that are correlated with *XIST* in MVC study participants, are more differentially expressed in women that develop CPTP and PTSS vs recover following MVC than any other set of eighteen genes.

Gene set enrichment analysis using Camera[6]

	# of genes	Direction*	P value
CPTP	18	Up	3.8×10^{-4}
PTSD	18	Up	5.4×10^{-6}

*direction of expression of genes in women who develop CPTP and PTSD relative to expression of the same genes in women who recover following MVC

FIGURE 5. Twelve of eighteen X chromosome genes that are positively correlated with *XIST* in women following MVC, are expressed at higher levels in women who develop CPTP and/or PTSS than in those who recover. Three examples of these gene transcripts are shown below.



RESULTS

- African American women age 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.

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