The Influence of microRNA on Chronic Pain Development after Motor Vehicle Collision May Be Sex-Dependent

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Multiple reports have demonstrated sex-dependent differences in the prevalence and severity of chronic pain disorders. The molecular mediators driving these differences remain poorly understood. MicroRNAs (miRNAs) are small non-coding RNA that regulate gene expression. Emerging data indicates that they may influence sex differences in disease outcomes and/or play sex-dependent roles in the pathogenesis of a variety of disease states, including persistent pain pathologies. Whether miRNA regulation of key pain transcripts contributes to sexual dimorphism in chronic pain outcomes after trauma exposure is not known.

HYPOTHESES
We hypothesized that a subset of miRNA would be predicted to preferentially regulate known pain transcripts/pathways and that many of these “pain miRNAs” would predict chronic pain development following motor vehicle collision trauma in a sex-dependent manner.

METHODS
In this study we used in silico and longitudinal human cohort data to test the hypothesis that miRNA play different roles in the pathogenesis of chronic pain after trauma exposure in men and women. We first used an unbiased in silico approach to identify miRNA that target gene transcripts that play an important role in pain processing (“pain genes”). Pain genes were identified using three published databases (n = 560 pain genes)1,2,3. Candidate miRNAs were determined via predicted binding to the 3’UTR of pain genes; Monte Carlo simulations (x10,000) consisting of randomly selected sets of genes were used to generate a background distribution of the number of predicted targets for each miRNA. This was then used to determine miRNA that preferentially target pain genes (“pain miRNAs”). In human studies, miRNA were identified via RNA seq from blood samples (n = 153) obtained from participants enrolled in a longitudinal study of chronic pain development following motor vehicle collision (MVC). Repeated measures linear mixed models were used to identify pain miRNAs that predicted pain outcomes 6 weeks, 6 months, and 1 year after MVC and to assess for miRNA that exhibited significant miRNA-sex interactions. Finally, we used DIANA miRPath4, an online algorithm, to identify specific KEGG pathways regulated by miRNA predicting pain persistent-MVC in a sex-dependent manner.

RESULTS
miRNA targeting the 3’UTR of one or more pain genes (range 1-161) were identified (n = 243) (Figure 1). Twenty-nine of these miRNA were predicted to be pain miRNAs (p < 0.05), including miRNA previously shown to be associated with stress and/or pain-related outcomes (e.g., miR-128, miR-132, miR-19 and miR-135) (Figure 2). One hundred and fifty three participants were enrolled in the immediate aftermath of MVC across 13 different Emergency Department sites in The United States (Figure 3). Details of this study population can be found in Table 1. Eleven (38%) of the pain miRNAs interacted with sex to predict chronic pain following MVC, including miR-103, miR-19, miR-181, and miR-129 (miRNA sex interaction, p < 0.05) (Figure 4). These 11 sex-dependent pain miRNAs are predicted to preferentially target pathways previously implicated in pain pathogenesis, such as the long term potentiation pathway (Figure 5).

CONCLUSIONS
We identified miRNA with specific/differential influence on pain regulation via in silico experiments. Using these pain miRNAs and human cohort data, we identified preliminary evidence that a number of miRNA predict and might play sex-dependent roles in post-MVC chronic pain development. Future studies will examine common regulatory elements driving differential miRNA expression in women and men following trauma exposure.

REFERENCES
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