The influence of microRNA on chronic pain development after motor vehicle collision may be sex-dependent

McLean S[1,2,3], Wu A[1,2], Gonzalez M[1,2], Harmon E[1,2], Zimny E[4], Lewandowski C[4], Hendry PL[5], Damiron K[6], Pearson C[7], Velilla MA[8], Swor R[9], Domeier R[10], Linnstaedt S[1,2]

From the [1]TRYUMPH Research Program, [2]Department of Anesthesiology, UNC-CH, [3]Department of Emergency Medicine, UNC-CH, [4]Department of Emergency Medicine Henry Ford Hospital; [5]Department of Emergency Medicine Shands Jacksonville Medical Center; [6]Department of Emergency Medicine Albert Einstein Medical Center; [7]Department of Emergency Medicine Detroit Receiving; [8]Department of Emergency Medicine Sinai Grace; [9]Department of Emergency Medicine William Beaumont Hospital; [10]Department of Emergency Medicine St. Joseph Mercy Health System

TRYUMPH Research Program
UNC Department of Anesthesiology
Trauma RecoverY: Understanding Mechanisms & Promoting Healing

INTRODUCTION

Multiple reports have demonstrated sex-dependent differences in the prevalence and severity of chronic pain disorders (e.g.[1,2]). The molecular mediators driving these differences remain poorly understood.

microRNA (miRNA) 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[3].

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 miR-hubs" 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)[4,5,6]. 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 miR-hubs"). 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 miR-hubs 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 miR-Path[7], an online algorithm, to identify specific KEGG pathways regulated by miRNA predicting pain post-MVC in a sex-dependent manner.

FIGURE 1. 243 miRNA are predicted to target at least one pain gene

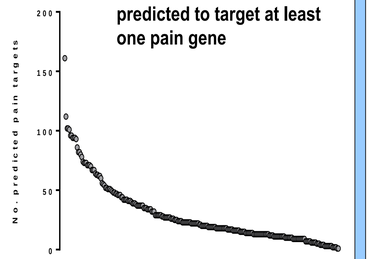


FIGURE 2. Twenty-nine miRNA identified as strong candidate pain miR-hubs

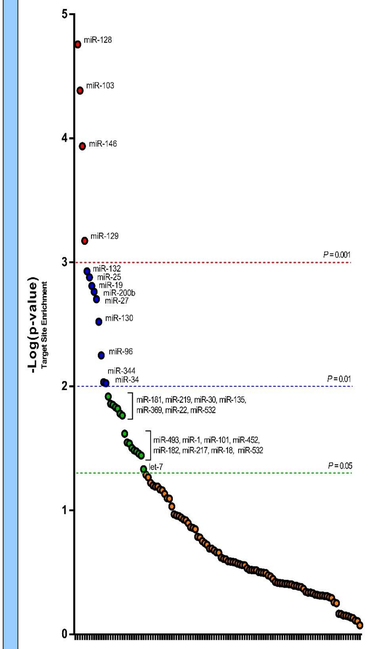


FIGURE 3. Study sites

TABLE1. Study participants

Characteristic	
Participants, n	153
Females, n (%)	95 (62)
Age, years, mean (SD)	35 (12)
Education, n (%)	
High school or less	56 (37)
Some college	68 (44)
College	22 (14)
Post-college	6 (4)
BMI, mean (SD)	29 (7)

FIGURE 4. Many pain miR-hubs predict chronic pain development following MVC in a sex-dependent manner.

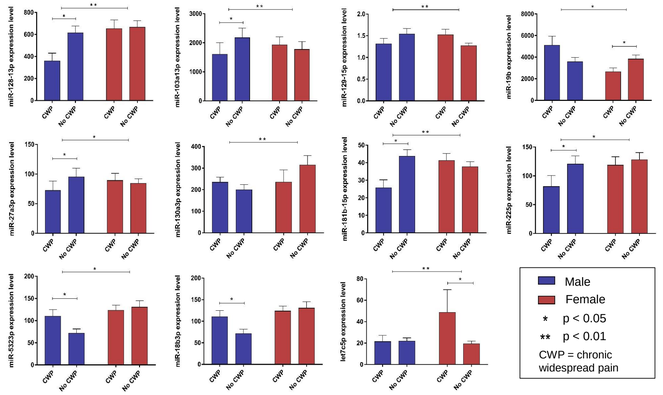
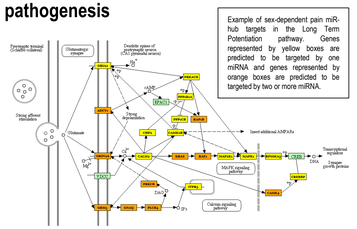


FIGURE 5. Sex-dependent pain miR-hubs are predicted to preferentially target pathways previously implicated in pain pathogenesis

Pathway	P value
Wnt signaling pathway	$8.7x10^{-24}$
ErbB signaling pathway	$1.7x10^{-22}$
Long Term Potentiation	$5.6x10^{-20}$
GnRH signaling pathway	$4.9x10^{-16}$
Neurotrophin signaling	$9.3x10^{-12}$
Axon guidance	$1.2x10^{-11}$



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 miR-hubs ($p < 0.05$), including miRNA previously shown to be associated with stress and/or pain-related outcomes (e.g., miR-128, miR-103, 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 miR-hubs 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 miR-hubs 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 miR-hubs 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

1. Mogil, JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nature Reviews Neuroscience 2012. Vol 13, Issue 12, Pages 859-866
2. Bartley, EJ, Fillingim, RB. Sex differences in pain: a brief review of clinical and experimental findings. British Journal of Anaesthesia 2013. Vol 111, Issue 1, Pages 52-58
3. Linnstaedt, SD, Walker MG, et al. MicroRNA circulating in the early aftermath of motor vehicle collision predict persistent pain development and suggest a role for microRNA in sex-specific pain differences.
4. Perkins JR, Lees J, Antunes-Martins A, Diboun I, McMahon SB, Bennett DL *et al.* PainNetworks: A web-based resource for the visualisation of pain-related genes in the context of their network associations. *PAIN®* 2013; **154**(12): 2586. e2581-2586. e2512.
5. Algynomics Pain Research Panel v2.0. http://www.algynomics.com/pdf/AlgynomicsPainPanel_v2_540.pdf, 2016, Accessed Date Accessed 2016 Accessed.
6. LaCroix-Fralish ML, Ledoux JB, Mogil JS. The Pain Genes Database: An interactive web browser of pain-related transgenic knockout studies. *Pain* 2007; **131**(1): 3. e1-3. e4.
7. Vlachos IS, Kostoulas N, Vergoulis T, Georgakilas G, Reczko M, Maragkakis M, Paraskevopoulou MD, Prionidis K, Dalamagas T, Hatzigeorgiou AG. DIANA miRPath v.2.0: investigating the combinatorial effect of microRNAs in pathways. Nucleic acids research 2012;40(Web Server issue):W498-504.

Funding by NIAMS R01AR056328, IBM Junior Faculty Grant, Future Leaders in Pain Grant, and The Mayday Fund