Sex-dependent expression of microRNA -19b predicts chronic widespread pain and posttraumatic stress disorder development following trauma exposure

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Trauma RecoverY: Understanding Mechanisms & Promoting Healing

INTRODUCTION

In humans, chronic widespread pain (CWP) and posttraumatic stress disorder (PTSD) are frequent sequelae of trauma that occur more commonly in women. One known trigger of CWP and PTSD is motor vehicle collision (MVC).[1,2] Molecular mechanisms mediating the development of CWP after MVC remain poorly understood. Using *in silico,* human, animal, and molecular studies, we sought to identify microRNA (miRNA), small regulatory RNA, that may contribute to CWP/PTSD vulnerability.

In preliminary studies, using an un-biased *in silico* approach to define miRNA that preferentially target pain/PTSD pathways, we identified miR-19 as a candidate regulatory hub. Interestingly, previous reports have shown that miR-19b (1) modulates the behavioral response to stress[3] and (2) is regulated by the estrogen receptor alpha[4]

HYPOTHESES

Based on the above data, we hypothesized that (1) circulating levels of miR-19b would predict CWP and PTSD following MVC trauma, (2) miR-19b expression is sex dependent and predicts CWP/PTSD outcomes in a sex- dependent manner, (3) miR-19b regulates gene transcripts known to play pathogenic roles in pain/PTSD.

METHODS

IN SILICO: We first used an unbiased approach to identify miRNA that target gene transcripts that play an important role in pain and PTSD processing. Pain and PTSD genes were identified using three published databases (n = 629 genes)[5,6,7]. Candidate miRNAs were determined via predicted binding to the 3'UTR of these genes (TargetScan); 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 distribution was then used to determine miRNA that preferentially target pain/PTSD genes. HUMAN: Next, we examined the association between miR-19b expression (identified via small RNA sequencing) assessed in blood samples (PAXgene RNA tubes, n = 153) collected in the emergency department in the immediate aftermath of motor vehicle collision (MVC) and the presence of CWP (ACR definition) and PTSD (Impact of Events Scale-Revised > 33) at 6 months using logistic regression analysis. ANIMAL: Male Sprague Dawley rats (n = 6 Male, 6 Female) were subjected to sound stress exposure as described previously[8]. Whole blood samples were collected using Qiagen RNAprotect Animal Blood Tubes before stress exposure, 24hours, and 2 weeks following stress; total RNA was isolated using RNeasy Protect Animal Blood Kits. miR-19b was quantified using TaqMan stem-loop RT-qPCR normalized to U87. MOLECULAR: 3'UTR constructs were made by amplifying 3'UTRs from genomic DNA using PCR, and inserting downstream of a Firefly Luciferase gene in pL-SV40-Fluc using Not1 and EcoR1 restriction enzyme sites. Mutations to predicted binding sites within the cloned 3'UTR were introduced via primers using two step PCR. In dual luciferase reporter assays, miR-19b binding to the specified 3'UTRs was quantified by measuring the level of Luciferin protein produced in HEK293T cells 72 hours after transfection with 20fmol pL-SV40-Rluc (transfection control), 20fmol pL-SV40-Fluc-3'UTR, and 10nM miR-19b or control mimic.

FIGURE 1. *In silico* analyses indicate that miR-19 is a strong candidate regulatory hub for both CWP and PTSD

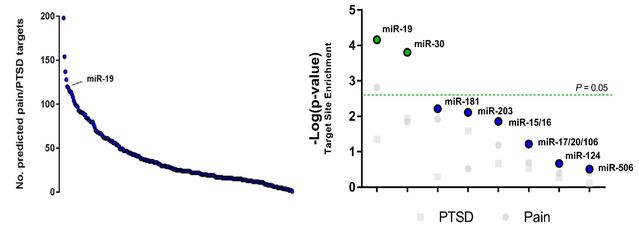


TABLE 1 and FIGURE 2. Participants and Study sites

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)

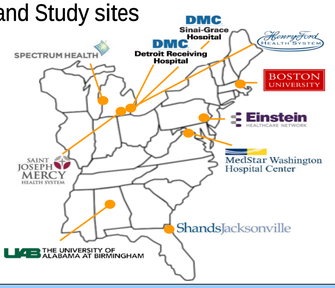


TABLE 2 and FIGURE 3. Circulating miR-19b expression levels predicts CWP and PTSD development following MVC and does so in a sex dependent manner

	CWP		PTSD	
Variable[a]	OR (95% CI)[b]	p value	OR (95% CI)[b]	p value
miR-19b	0.20 (0.04, 0.95)	0.043	0.16 (0.04, 0.75)	0.020
Sex	<0.01 (0.00, 0.35	0.027	<0.01 (0.00, 0.06)	0.009
miR-19b*sex	16.46 (1.53, 177.67)	**0.021**	35.67 (3.02, 422.02)	**0.005**
Age	1.01 (0.98, 1.05)	0.417	0.99 (0.95, 1.03)	0.959

[a]Site was also included in the model as a categorical variable. [b]OR = odds ratio, CI = confidence interval

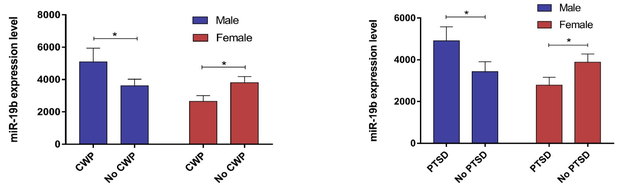


FIGURE 4. Circulating miR-19b expression differs in male and female rats and changes in a sex dependent manner following sound stress exposure

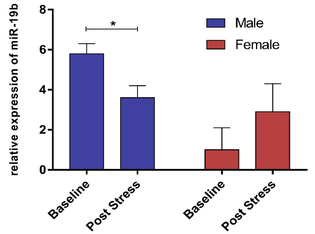
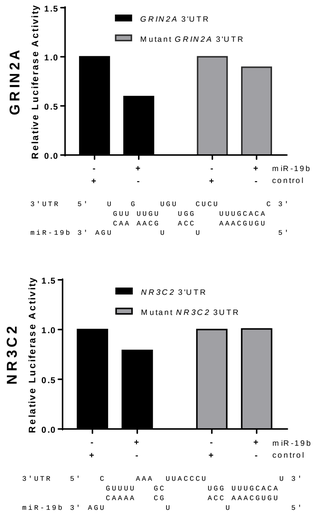


FIGURE 5. *GRIN2A* and *NR3C2* are directly regulated by miR-19b



RESULTS

IN SILICO: *In silico* analyses indicated that miR-19 targets more genes implicated in pain/PTSD pathogenesis than would expected based on chance (n = 112/629, p < 0.05, Figure 1).

HUMAN: miRNA samples were obtained from study participants enrolled at 10 US Emergency Department sites (Figure 2). All individuals were African Americans between the ages of 18-65, study participant characteristics are shown in Table 1. Circulating expression levels of miR-19b predicted both CWP and PTSD in a sex-dependent manner (Table 2). In males, higher expression of miR-19b predicted CWP/PTSD. In contrast, in females, lower expression of miR-19b predicted CWP/PTSD (Figure 3).

ANIMAL: miR-19b expression was sex-dependent. Following sound stress exposure, miR-19b expression levels decreased in male animals and increased in female animals (Figure 4).

MOLECULAR: Among initial pain/PTSD genes tested, the glutamate receptor 2A (*GRIN2A*) and Mineralcorticoid receptor (*NR3C2*) are direct targets of miR-19b (Figure 5). Many additional genes predicted to be targeted by miR-19b are in the circadian rhythm pathway (e.g. *RORA*, *CLOCK*, *PER2*, *OPN4*). Testing for miR-19b binding to these additional targets is underway.

CONCLUSIONS

miR-19b expression influences the development of chronic widespread pain and PTSD after motor vehicle collision. Sex-dependent differences in miR-19b suggest that this miRNA may mediate sex differences in CWP/PTSD vulnerability after common traumatic events.

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