Common genetic variations in *ADRA2A* that influence stress-induced analgesia might be mediated by microRNA-34a

Linnstaedt SD[1,2], Harmon E[1,2], Walker MG[1,2], Levine J[3], Riker K[1,2], Nyland JE[3], Hu, JM[1,2], Zimny E[4], Rossi C[4], Swor RA[5], Jones JS[6], Diatchenko L[7], Bortsov AV[1,2], Peak DA[8], McLean SA[1,2,9]

From the [1]TRYUMPH Research Program, [2]Department of Anesthesiology, University of North Carolina, Chapel Hill, North Carolina, [3]Department of Battlefield Pain Research, USAISR, [4]Forensic Nursing Program, Medicine, Cone Health System, Greensboro, North Carolina, [5]Emergency Medicine, William Beaumont Hospital, Royal Oak, Michigan [6]Department of Emergency Medicine, Spectrum Health Butterworth Campus, Grand Rapids, Michigan, [7]The Allan Edwards Centre for Research on Pain, McGill University, Montreal, Canada, [8]Emergency Medicine, Massachusetts General Hospital, Boston, Massachusetts, [9]Department of Emergency Medicine, University of North Carolina, Chapel Hill, North Carolina

INTRODUCTION

Traumatic stress is common, but the precise molecular mechanisms influencing acute musculoskeletal pain (MSP) severity after stressful events such as motor vehicle collision (MVC) and sexual assault (SA) remain poorly understood.

Adrenergic alpha 2A receptors (α2A-AR) play an important role in spinal cord descending pathways which inhibit dorsal horn nociceptive transmission, and are also important to preventing stress-induced hyperalgesia in peripheral sensory neurons[1].

The influence of α2A-AR on MSP outcomes in humans experiencing traumatic stress has not been assessed. If α2A-AR activation influences post-traumatic MSP in humans, then inherited differences in its encoding gene, *ADRA2A*, should be associated with individual differences in acute MSP severity.

Bioinformatics analyses indicate that a particular *ADRA2A* single nucleotide polymorphism (SNP), rs3750625, occurs in the seed binding region of miR-34a, a miRNA known to affect pain and stress responses[2,3].

One common mechanism by which genetic polymorphisms influence cellular function is by altering miRNA seed binding regions[4], thereby disrupting miRNA regulation of the cellular transcriptome and, subsequently, the cellular proteome.

HYPOTHESES

Based on the above data, we hypothesized that (1) *ADRA2A* rs3750625 allele copy number influences acute MSP severity following MVC and SA, (2) miR-34a binds the 3'UTR of the *ADRA2A* transcript and rs3750625 subsequently affects miR-34a binding, (3) miR-34a and *ADRA2A* expression levels change in key tissues involved in stress-induced hyperalgesia in an animal model of stress exposure.

METHODS

IN SILICO: The miRdSNP online algorithm was used to identify whether rs3750625 was predicted to affect miRNA binding. The UCSC genome browser was used to determine species conservation. RNA hybrid was used to predict the secondary structure of the miR-34a-*ADRA2A* binding event. HUMAN: Sociodemographic characteristics of the sample were summarized using standard descriptive statistics. General linear models were used to evaluate the association between rs3750625 with acute MSP outcomes and for potential sex x rs3750625 and stress x rs3750625 interactions, adjusting for potential confounding by age and study site. 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-34a 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-34a or control mimic. ANIMAL: Male Sprague Dawley rats (n = 6) were subjected to swim stress exposure as described previously. Twenty-four hours following stress/sham exposure, rats were sacrificed and the following tissues were collected: adrenal glands (AG), peripheral nerves (PN), and dorsal root ganglion (DRG) from L2-L5. RNA was collected using Trizol purification and RNA concentration was measured using a NanoDrop 2000 (Thermo Scientific); RNA levels were detected using TaqMan RT-qPCR.

FIGURE 1. rs3750625 is in the seed binding region of miR-34a

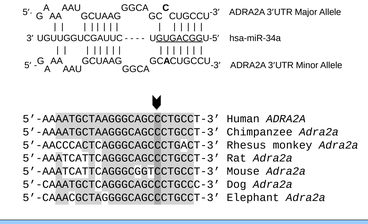


TABLE 1. Study Participants

Characteristic	MVC	SA
Enrolled, n	948	84
Age, years, mean (SD)	36 (13)	26 (8)
Females, n(%)	575 (61)	84 (100)
Ethnicity		
European American	948 (100)	54 (64)
African American	-	30 (36)
Education, n(%)		
8-11 years	42 (4)	11 (13)
HS	184 (19)	16 (19)
Post-HS training (not college)	57 (6)	1 (1)
Some college	311 (33)	43 (51)
College	237 (25)	9 (11)
Post-college	113 (12)	3 (4)
ED pain, 0-10 NRS, mean(SD)	5.5 (2.4)	6.8 (2.9)

FIGURE 2. Acute pain following trauma is more severe in individuals with the minor allele at *ADRA2A* rs3750625 who also reported stress in the early aftermath of trauma exposure

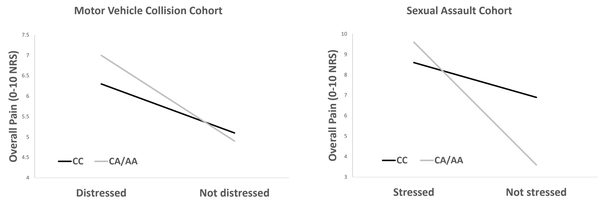


FIGURE 3. The efficiency of miR-34a binding to ADRA2A is dependent on the allele at rs3750625

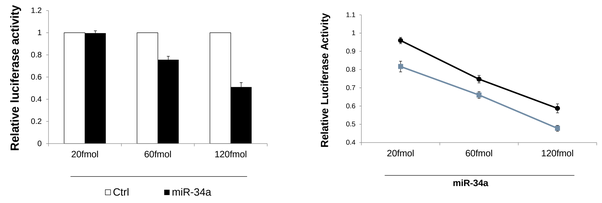
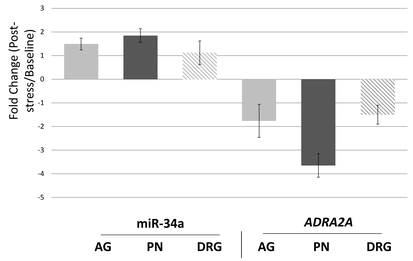


TABLE 2. Influence of rs3750625 on acute pain severity following MVC and SA depends on the level of peritraumatic distress

	Motor Vehicle Collision		Sexual Assault	
Variable[a]	F	p value	F	p value
Age	3.83	0.051	<0.001	0.999
Sex	0.037	0.947	N/A	N/A
Peritraumatic distress[b]	34.19	<0.001	0.011	0.918
rs3750625[c]	3.224	0.073	5.432	0.007
rs3750625 * sex	0.102	0.750	N/A	N/A
rs3750625 * distress	4.103	**0.043**	5.467	**0.007**

FIGURE 4. miR-34a and ADRA2A are co-expressed in rat tissues relevant to pain and stress and their expression levels change in response to forced swim stress exposure



RESULTS

IN SILICO: *In silico* analyses indicated that *ADRA2A* rs3750625 is located in the seed binding region for miR-34a (Figure 1, Top). The minor allele is predicted to create and A-U base-pair between miR-34a and *ADRA2A*, increasing seed binding affinity between the two bases. However, this position is unpaired if miR-34a binds the major allele. The seed binding region is highly conserved amongst mammals (Figure 1, Bottom).

HUMAN: Characteristics of the study populations are outlined in Table 1. In initial general linear models, the influence of *ADRA2A* rs3750625 on acute MSP severity after both MVC and SA depended on the level of peritraumatic stress (Table 2). Individuals who reported higher levels of peritraumatic stress in the early aftermath of SA and had one or more copies of the minor allele (CA/AA) experienced more severe acute MSP following SA than individuals homozygous for the major allele (CC = 9.3 vs CA/AA = 8.4, p = 0.020, Figure 2, Right). The same direction and size of effect was observed in the MVC cohort among those who reported higher levels of peritraumatic stress during and after the MVC, although this difference did not reach statistical significance (CC = 7.1 vs CA/AA = 6.3, p = 0.120, Figure 2, Left).

MOLECULAR: miR-34a binding to the 3'UTR of *ADRA2A* caused a dose-dependent decrease in luciferase production (Figure 3, Left). Additionally, decreased luciferase production was observed in the presence of the minor allele vs the major allele at all concentrations of miR-34a (Figure 3, Right).

ANIMAL: miR-34a and *ADRA2A* are co-expressed in rat tissues relevant to pain and stress and their expression levels change in response to forced swim stress exposure in peripheral nerve tissue (Figure 4).

CONCLUSIONS

Distressed individuals with the *ADRA2A* rs3750625 minor allele experience increased acute MSP following MVC or SA. Current data suggests that this might be due to more efficient binding of miR-34a to the 3'UTR of the *ADRA2A* gene transcript when the minor allele is present.

REFERENCES

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