



A genetic variant in ADRA2A predicts extent of acute pain after motor vehicle collision

Linnstaedt S^{1,2}, Bortsov A^{1,2}, Walker M^{1,2}, Swor R⁶, Jones J⁴, Lee D⁷, Peak D⁵, Domeier R⁹, Rathlev N⁸, McLean S^{1,2,3}

From the ¹TRYUMPH Research Program (JU, SL, SM), ²Department of Anesthesiology (DO, JU, SL, SM), and ³Department of Emergency Medicine (SM), University of North Carolina, Chapel Hill, NC; ⁴Department of Emergency Medicine Spectrum Health Butterworth Campus (JJ); ⁵Department of Emergency Medicine Massachusetts General Hospital (DP); ⁶Department of Emergency Medicine William Beaumont (RS); ⁷Department of Emergency Medicine North Shore University Hospital (DL); ⁸Department of Emergency Medicine Bay State Medical Center (NR); ⁹Department of Emergency Medicine St. Joseph Mercy Hospital (RD); ¹⁰Department of Emergency Medicine Shands Jacksonville



INTRODUCTION

More than 50 million individuals experience motor vehicle collision (MVC) per year worldwide. As with other stress/trauma exposures, the molecular mechanisms mediating inter-individual variation in acute pain experiences between individuals experiencing MVC remain poorly understood.

A deficit of descending pain inhibitory mechanisms has been implicated in the spread of pain and pathogenesis of widespread pain.¹ Adrenergic alpha 2A receptors (ADRA2A) 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.² These data suggest that genetic variants influencing the function of ADRA2A may contribute to individual differences in pain extent and severity after MVC.

The precise mechanisms by which most single nucleotide polymorphisms (SNPs) affect pain outcomes are not understood. Increasing evidence suggests that microRNA (miRNA), small RNA regulators of gene expression, may often play an important role. Understanding molecular mechanisms mediating pain outcome differences is important because it creates the opportunity to develop novel pain interventions.

HYPOTHESIS/RATIONALE

In this study we evaluated the association between a common SNP within the ADRA2A gene, rs3750625, and the spread (extent) and severity of acute pain in response to MVC. We hypothesized that rs3750625 predicts acute pain severity and extent in the ED. In addition, because bioinformatics analyses suggest that rs3750625 alters a binding site for miR-34a, a miRNA involved in the stress response and in pain processing,^{3,4} we hypothesized that the molecular effect of rs3750625 is mediated by miR-34a.

METHODS

Individuals (n = 948) between the ages of 18 and 65 presenting to one of eight emergency departments (EDs) in four no-fault insurance states for evaluation after MVC who did not have a fracture or require hospital admission were enrolled. Pain extent was assessed at 20 body regions on a 0-10 Numeric Rating Scale (NRS). Number of body regions with pain was defined as the number of body regions with a pain score of 1 or more. Overall body burden of pain was assessed by summing 0-10 NRS pain scores in the ED in each of the 20 regional pain scale areas (range 0-200). DNA was collected in PAXgene tubes at the ED; genotyping was performed using the Sequenom platform. miRNA binding was assessed using a dual luciferase reporter assay. The ADRA2A 3' untranslated region (UTR) was cloned from genomic DNA and inserted downstream of the firefly luciferase gene. Site directed mutagenesis was used to change the major to the minor allele. Luciferase activity was measured on a luminometer 48 hours after co-transfection of reporter constructs and a miR-34a expression vector into HEK293T cells.

TABLE 1. Study characteristics, data collection sites

Variable	n = 948
Mean age (yrs)	35.4 (± 13.3)
Female (n, %)	575 (61)
Education (n, %)	
≤ HS	227 (34)
some college	369 (39)
≥ college	351 (37)
Income, n (%)	
<20K	117 (12)
20K to 40K	176 (19)
40K to 80K	277 (29)
>80K	273 (29)
Collision type, n (%)	
Front end	439 (46)
Rear end	341 (36)
Other	168 (18)



TABLE 2. Individuals with one or more A alleles at rs3750625 experience a greater extent of pain and a greater overall body burden of pain after MVC

		rs3750625 genotype		p-value
		C/C	C/A + A/A	
Mean number of pain regions in ED ^a	Mean	4.36	5.19	0.035
	95%CI	(4.10, 4.61)	(4.44, 5.94)	
Body burden of pain in the ED ^b	Mean	23.75	29.11	0.037
	95%CI	(21.84, 25.66)	(24.22, 34.01)	

^aAdjusted for sex and study site. N = 942

^bAdjusted for sex, study site, income, and relationship status. N = 842

FIGURE 1. Multiple species conservation of nucleotides in the miR-34a binding domain of ADRA2A

5'-AATGCTAAGGGCAGCCCTGCC-3' Human ADRA2A
 5'-CCCACCTCAGGGCAGCCCTGAC-3' Rhesus Adra2a
 5'-ATCATTTCAGGGCGGTCCTGCC-3' Mouse Adra2a
 5'-AATGCTCAGGGCAGCCCTGCC-3' Dog Adra2a
 5'-AACGCTAAGGGCAGCCCTGCC-3' Elephant Adra2a

FIGURE 2. rs3750625 is located in the 3'UTR of ADRA2A and is predicted to increase binding with the critical seed region of miR-34a

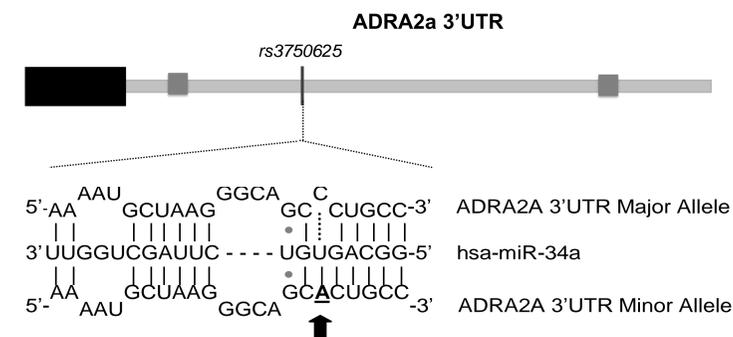


FIGURE 3. In a reporter binding assay, miR-34a binds to the 3'UTR of ADRA2A and represses luciferase activity in a dose dependent manner

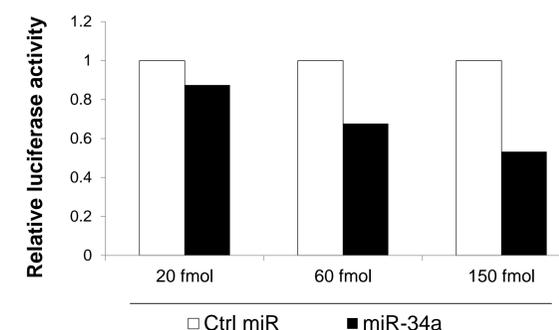
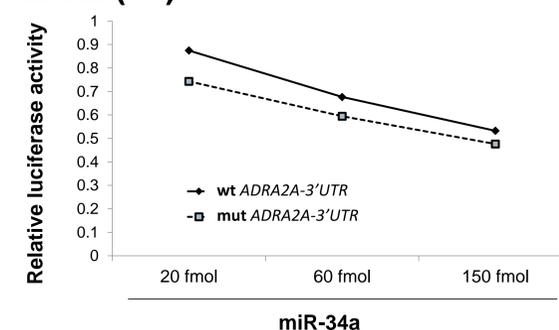


FIGURE 4. The ADRA2A 3'UTR containing the minor allele (mut) is repressed to a greater extent than the ADRA2A 3'UTR containing the major allele (wt)



RESULTS

In regression models adjusted for sex and study site, the minor (A) allele of rs3750625 (n = 91/942, 9.7%) was associated with a significantly higher mean number of pain regions in the ED (Table 2). In regression models adjusted for sex, study site, income, and relationship status, the minor allele (A) of rs3750625 (n = 81/842, 9.6%) was associated with a significantly higher severity of pain in the ED (Table 2).

A potential binding site for miR-34a within the ADRA2A gene is highly conserved across five species, supporting its potentially important role in biologic function (Figure 1).

This binding site is located in the 3'UTR of ADRA2A. rs3750625 is located within this binding region; bioinformatics analyses predict that the A allele strengthens miR-34a binding and results in greater miR-34a repression of ADRA2A (Figure 2).

Results of preliminary in vitro testing (average of two independent experiments performed in triplicate) suggest that miR-34a binds and represses ADRA2A 3'UTR in a dose dependent manner (Figure 3) and that the minor allele associated with increased extent and severity of pain after MVC creates a more robust binding site for miR-34a (Figure 4).

CONCLUSIONS

ADRA2A SNP rs3750625 is associated with pain extent and severity in the acute aftermath of MVC. Bioinformatics analyses and follow-up in vitro testing suggest that the influence of this SNP on acute pain extent and severity after MVC may be mediated by its effect on the function of a miRNA, miR-34a. Further studies are required to show the extent of miR-34a regulation of ADRA2A and the functional consequences of this interaction with respect to acute pain characteristics following trauma/stress exposure. Understanding molecular mechanisms mediating acute pain outcomes has the potential to lead to the development of novel interventions that improve pain outcomes.

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