# The *ADRA2A* genetic variant rs3750635 influences extent and severity of acute pain after motor vehicle collision and may do so by regulating microRNA function

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## INTRODUCTION

Adrenergic alpha 2A receptors (ADRA2A) play an important role in spinal cord descending pathways which inhibit acute pain transmission.[1] Experiments in pre-clinical models have demonstrated a role for ADRA2A in the inhibition of pain following stress exposure.[2] However, the role of ADRA2A in the inhibition of pain following stress exposure in a human cohort and mechanisms of ADRA2A regulation have not been explored. 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 among patients presenting to the emergency department (ED) after motor vehicle collision (MVC).

## HYPOTHESES

rs3750625 is a *ADRA2A* SNP that bioinformatics analyses suggest alters a binding site for miR-34a, a miRNA involved in the stress response and in pain processing.[3,4] We hypothesized that genetic variants in *ADRA2A* rs3750625 predict acute pain severity and extent after MVC. In addition, we hypothesized that the molecular effect of *ADRA2A* rs3750625 is mediated, at least in part, by miR-34a.

## METHODS

Individuals (n=948) between the ages of 18 and 65 presenting to one of eight 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 1-10 Numeric Rating Scale (NRS). Number of body regions with pain was defined as the number of body regions with 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 the ED using PAXgene tubes; 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. qPCR was used to quantify *ADRA2A* gene expression and miR-34a levels in neuroblastoma cell lines.

**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 *ADRA2A* rs3750625 experience a greater extent of pain and a greater overall body burden of pain after MVC**

| | | rs3750625 genotype | | |
|---|---|---|---|---|
| | | C/C | C/A + A/A | p-value |
| **Mean number of pain regions in ED*** | Mean 95%CI | 4.78 (4.22, 5.33) | 6.22 (4.78, 7.67) | 0.047 |
| **Body burden of pain in the ED*** | Mean 95%CI | 26.09 (22.94, 29.24) | 38.81 (30.59, 47.02) | 0.002 |

*Adjusted for age, sex, study site, education, and BMI. N = 929

**FIGURE 1. rs3750625 is located in the 3'UTR of *ADRA2A* and is predicted to increase binding with the seed region of miR-34a**

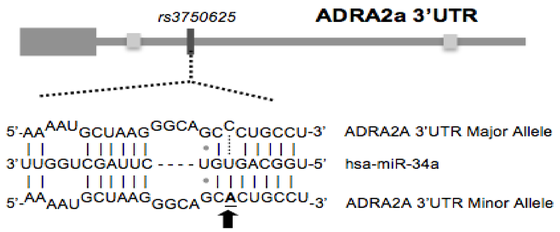



**FIGURE 2. Multiple species conservation of nucleotides in the miR-34a binding domain of *ADRA2A***

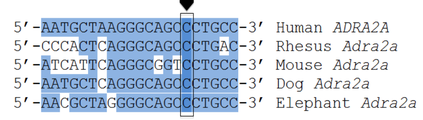



**FIGURE 3. In a reporter binding assay, miR-34a binds to the 3'UTR of *ADRA2A* and represses luciferase activity in a dose dependent manner**

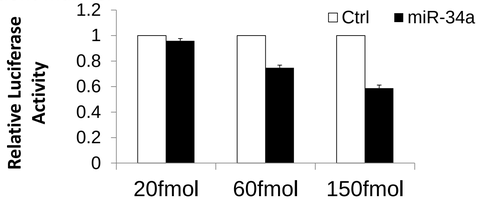



**FIGURE 4. The *ADRA2A* 3'UTR containing the minor allele (mut) is repressed to a greater extent than the *ADRA2A* 3'UTR containing the major allele (wt)**

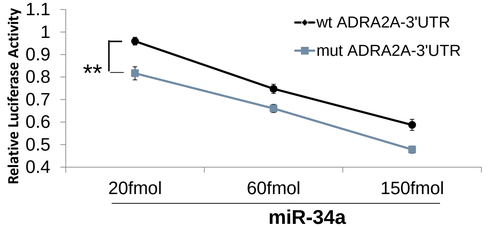
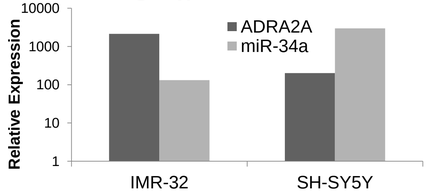



**FIGURE 5. miR-34a and *ADRA2A* mRNA are both expressed in adrenergic-type neuroblastoma cell lines**




## RESULTS

- The minor (A) allele of *ADRA2A* rs3750625 (n = 91/942, 9.7%) was associated with a significantly higher mean number of pain regions in the ED and a significantly higher severity of pain in the ED (Table 2).
- rs3750625 is located in the 3'UTR of *ADRA2A* and bioinformatics analyses predict that miR-34a could bind in this region and that the (A) allele strengthens seed base pairing (Figure 1).
- The potential binding site for miR-34a within the *ADRA2A* gene is highly conserved across five species (Figure 2), supporting its potentially important role in biologic function.
- In vitro testing with luciferase assays show 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 (**p < 0.005 at all concentrations of miR-34a, using Mann Whitney U tests) (Figure 4).
- Adrenergic neuroblastoma cell lines IMR-32 and SH-SY5Y express both *ADRA2A* and miR-34a (Figure 5).

## 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 show that the influence of this SNP 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 exposure to a stressful/traumatic event.

## REFERENCES

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