

Genetic variant rs3750625 in the 3'UTR of ADRA2A affects the sleep quality of patients in the ICU by promoting miR-34a binding to ADRA2A

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Abstract. Poor sleep is very common in patients in the ICU and hence, sleep quality is considered an important aspect of intensive care; however, the underlying mechanisms of poor sleep in patients in the ICU remain unknown. In this study, we aimed to explore the role of rs3750625, which is located in the 3'UTR of adrenoceptor alpha 2A (ADRA2A), in sleep quality. For this purpose, luciferase assay was conducted to investigate the association between miR-34a and ADRA2A, and the effect of rs3750625 on the binding affinity between miR-34a and ADRA2A was examined. RT-qPCR and western blot analysis were carried out to examine the regulatory association between miR-34a and ADRA2A. The differences in sleep time and efficiency were compared between groups carrying the AC and CC genotypes of rs3750625, respectively. According to the results from an online search, miR-34a could directly bind to the 3'UTR of ADRA2A, and such binding was confirmed by the observation that miR-34a inhibited the luciferase activity of major or minor ADRA2A 3'UTR in a dose-dependent manner in HCN-1A and U251 cells. In addition, the ADRA2A protein and mRNA levels in the HCN-1A and U251 cells were evidently decreased following transfection with miR-34a precursors. Notably, patients in the AC group exhibited a similar level of miR-34a mRNA expression compared with patients in the CC group; however, the ADRA2A mRNA and protein levels in the CC group were significantly increased in comparison with those in the

AC group. In addition, the sleep time and sleep efficiency in the CC group were much higher than those in the AC group. Furthermore, the mean arterial pressure (MAP) values in both the AC and CC groups remained stable from 22:00 to 08:00, and the respiratory rates in both groups were quite similar. However, the heart rate of patients in the CC group was much lower than that of patients in the AC group. On the whole, the findings of this study suggest that the genetic variant rs3750625 in the 3'UTR of ADRA2A affects the sleep quality of patients in the ICU by promoting the binding of miR-34a to ADRA2A, and hence it may serve as a novel biomarker for the prediction of the sleep quality of patients in the ICU.

Introduction

In recent years, the study of sleep disorders in critically ill patients has drawn increasing attention (1). In these patients, the amount of sleep is reduced and the pattern of sleep is fragmented without following the normal circadian rhythm. In addition, these patients have a higher proportion of sleep in the N1-N2 stages, whereas the amount of rapid eye movement (REM) in these patients is decreased. Since REM and sleep in the N3 stage play an important role in a wide range of physiological functions involving the immune, respiratory, endocrine, cardiovascular and central nervous systems, the lack of REM and N3 sleep in patients in the intensive care unit (ICU) significantly affects their recovery (1). For example, inadequate sleep has been shown to cause post-traumatic stress disorder, non-invasive ventilation failure and delirium (2-4).

It is known that α_2 agonists can result in a loss of consciousness, a loss of righting reflex (LORR), presumed surrogate and sedation (5). On the other hand, the activity of locus coeruleus (LC) neurons is decreased during sleep and begins to increase before an individual wakes up, indicating that these neurons are associated with consciousness (6). Notably, although the selective stimulation of LC neurons induces waking, their inhibition cannot help sleep (7). In addition, in mice lacking the ability to produce noradrenalin (NA), an agonist of the α_2 (ADRA2A) receptor, the administration of dexmedetomidine can still result in LORR (5). Nevertheless, in the absence

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of its endogenous ligand, the response of $\alpha 2A$ -receptors can become hypersensitized in the long-term, making it difficult to interpret the results (5).

As a class of small and non-coding RNAs containing 19-25 nucleotides, microRNAs (miRNAs or miRs) have been shown to mediate the expression of a wide range of genes both in animals and plants (8). In addition, a number of miRNAs are involved in various cellular processes, such as metabolism, stress response, stem cell renewal, embryonic development, cell proliferation, apoptosis and differentiation (9). Given these critical functions of miRNAs, it is reasonable to expect that the abnormality of miRNA pathways and miRNA synthesis can result in several human diseases, including obesity, cardiovascular diseases, cancer, psoriasis, schizophrenia, chronic hepatitis, acquired immune deficiency syndrome (AIDS) and diabetes (10-15). In addition, single-nucleotide polymorphisms (SNPs) in the 3'untranslated region (3'UTR) of genes can alter the binding of miRNAs and affect the expression of target proteins, thus influencing the pathogenesis of a number of diseases (16). A previous study on healthy subjects demonstrated that a 3'UTR polymorphism in adrenoceptor alpha 2A (ADRA2A) mediates the onset of autonomic responses upon environmental and physiological stimulation, while the polymorphisms of ADRA2A can also be used to predict the responses to induced pain (17). In addition, the correlation between rs3750625, a 3'UTR SNP in ADRA2A, and acute musculoskeletal pain (MSP) has been evaluated among individuals experiencing sexual assault and motor vehicle collision (MVC) (18,19). Furthermore, results of bioinformatics analyses have suggested that rs3750625 is located in the seed binding region of miR-34a, which is known to mediate stress and pain (20).

ADRA2A has been reported to be associated with sleep quality (21). Since miR-34a acts as a direct regulator of ADRA2A, the presence of rs3750625 has been shown to interrupt the interaction between miR-34a and ADRA2A (18,22). Therefore, in this study, we evaluated the regulatory association between miR-34a and ADRA2A, as well as the association between rs3750625 and sleep quality in patients in the ICU.

Materials and methods

Collection of blood samples. The Human Research Ethics Committees of Dongguan Houjie Hospital approved this research. Written informed consent was obtained from all patients or their first-degree relatives prior to the onset of this research. The research process was in conformity with the latest version of Declaration of Helsinki. In this study, peripheral blood samples were collected from 38 subjects (the ethnicity of all participants was Han; sex: Female, 18 male, 20 mean age, 50 ± 0.6 years old) enrolled from patients who stay in ICU for at least 3 days. The inclusion criteria were the following: i) An age between 18 and 65 years; ii) a stay in the ICU of at least 3 days, but the use of mechanical ventilation not required; iii) stable hemodynamic parameters without sedative treatment. The exclusion criteria were the following: i) Patients diagnosed with hypoxic-ischemic encephalopathy; ii) patients diagnosed with psychiatric disorders; iii) patients diagnosed with hemodynamic instability; iv) patients diagnosed with severe abnormalities in the cardiac conduction system; v) patients diagnosed with ventricular dysfunction;

vi) patients diagnosed with liver failure and a bilirubin level >100 mmol/l; vii) patients diagnosed with neurological injury or diseases that may influence sleep quality; viii) patients with a scale of Glasgow Coma score of <11 ; and ix) patients with an acute physiology score portion of the Acute physiology and Chronic Health evaluation II (APACHE II) score of >15 .

Genotyping. Genomic DNA was isolated from abdomen tissue samples using a DNA extraction kit (Shunhua Bioengineer Co. Ltd.) and amplified using PCR. An ExoSAP-IT purification kit (USB) was utilized to purify the PCR products, which were then sent to a core facility for sequencing.

Sleep quality measurement. During the entire course of this study, the light in the ward was maintained at a minimum level so as to not affect the sleep of the subjects. For subjects in the 'no sedation' group of this study, they had to be removed from the study if they suffered from sleep disorders and required sedation. For all the remaining subjects, a measurement of bispectral index (BIS) was carried out by recording their sleep time and sleep depth continuously using a Covidien BIS VISTA monitor. In addition, the level of sedation (if applicable) and the depth of sleep were graded based on the BIS index, which scored EEG signals from 0 to 100. A score of 65-85 indicated a patient was in sedation or sleep, while a score of 85 to 100 suggested the patient was awake. In addition, the Richmond Agitation-Sedation Scale (RASS) score (23), the duration of consciousness and sleep, blood pressure, heart rate, respiratory rate, oxyhemoglobin saturation, and the duration of total sleep of each subject were examined every 2 h. The ratio between the duration of effective sleep and the duration of total sleep was defined as sleep efficiency.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). TRIzol reagent (Invitrogen; Thermo Fisher Scientific) was utilized to extract total RNA from HCN-1A and U251 cells and blood samples. The quality of the RNA was determined by measuring its absorbance with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). A Sprint Power Script Pre Primed Single Shots kit (Clontech) was utilized to synthesize first-strand ADRA2A cDNA from total RNA using the following reaction conditions: 5 cycles of 30 sec at 94°C , 30 sec at 60°C and 60 sec at 68°C . A TaqMan hsa-miR-34a amplification kit (Applied Biosystems) was used to amplify the cDNA, which was then measured on an Applied Biosystems Real-Time PCR System (Applied Biosystems). The real-time PCR reaction mixture contained $4.0\ \mu\text{l}$ (a total of 40 ng) of ADRA2A cDNA, $10\ \mu\text{l}$ of 2X gene expression master mix, $1\ \mu\text{l}$ of 20X Gene Expression Assay buffer and $5\ \mu\text{l}$ of RNase-free water. The 3'UTR of ADRA2A was amplified with primers, including ATGATGCTCGAGACTCAGAAACCCGGGCGC (forward) and ATGATGAATTCCCATATAAATCAGATGT TCCAGAG (reverse). GAPDH and U6 were used as internal controls to normalize the relative expression of miR-34a and ADRA2A, respectively. The relative expression of miR-34a and ADRA2A mRNA was calculated using the $2^{-\Delta\Delta\text{Cq}}$ method (24). Each experiment was carried out at least 3 times.

Cells, cell culture and transfection. The HCN-1A and U251 cell lines were obtained from the American Type Culture

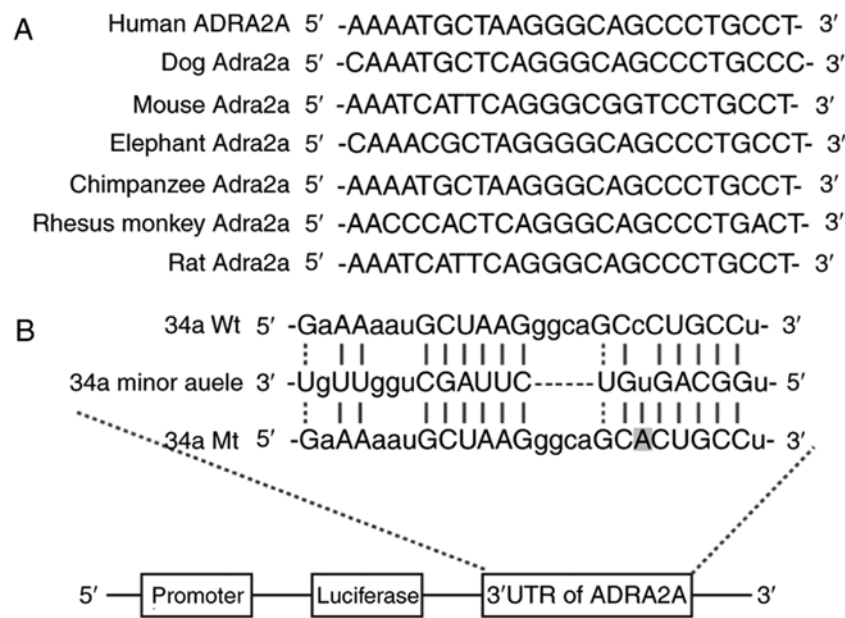


Figure 1. ADRA2A rs3750625 in the seed region containing putative binding site of miR-34a. (A) 'Seed sequence' in the 3' UTR of ADRA2A was highly conserved among species. (B) Single site or 'seed sequence' in ADRA2A 3'UTR were mutated, and major, minor or mutant ADRA2A 3'UTR were inserted into pGL3 vector immediately located downstream of luciferase gene. ADRA2A, adrenoceptor alpha 2A.

Collection (ATCC). For transfection experiments, the cells were seeded in 24-well plates and cultured for 24 h at 37°C and 5% CO₂ in a RPMI medium containing 10% heat-inactivated fetal bovine serum (FBS), 50 U/ml streptomycin/penicillin and 2 mM glutamine (Euroclone). When the cells reached 50-80% confluence, they were transfected with 30 nM miR-34a precursors, ADRA2A siRNA or a scramble control (RiboBio) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific). Subsequent experiments were accordingly performed after 48 h of transfection. Each experiment was performed at least 3 times.

Luciferase assay. The 3'-UTR of ADRA2A, which was found to contain the putative binding site of miR-34a by use of online database miRDB (<http://mirdb.org/>), was amplified using PCR. Subsequently, the PCR products (the major allele ADRA2A 3'UTR) were cloned into a pGL3 vector (Promega) and the correct sequence of the construct was confirmed by direct sequencing. In addition, a specific single-base mutation in ADRA2A 3'UTR (the minor allele ADRA2A 3'UTR), as well as a mutant ADRA2A 3'UTR was produced using a QuikChange[®] Lightning Site-Directed Mutagenesis kit (Agilent Technologies) and primers, including GCTAAG GGCAGCACTGCCTGCCCTC (forward) and GAGGGC AGGCAGTGCTGCCCTTAGC (reverse). Subsequently, both minor allele ADRA2A 3'UTR and mutant ADRA2A 3'UTR were inserted into pGL3 vectors (Promega), respectively, and the correct sequences of these constructs were also confirmed by direct sequencing. During the subsequent luciferase assay, HCN-1A and U251 cells were co-transfected with 0.4 µg of Firefly luciferase reporter constructs containing the major, minor or mutant ADRA2A 3'UTR, 0.02 µg of control vector containing a *Renilla* luciferase (Promega), and various concentrations of miR-34a precursors using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific). At 48 h post-transfection, a Dual-Luciferase Reporter Assay System (Promega) was

utilized to determine the luciferase activity of Firefly luciferase, which was then normalized to the luciferase activity of *Renilla* luciferase to obtain the final results. All tests were performed in triplicate.

Western blot analysis. RIPA buffer (Invitrogen; Thermo Fisher Scientific) supplemented with a protease inhibitor cocktail and phosphatase (Roche Diagnostics) was used to extract the total protein from HCN-1A and U251 cells. The lysate was then boiled for 10 min in 2-mercaptoethanol and 10% SDS-PAGE was used to separate the proteins. Subsequently, the proteins were transferred onto a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ) at 90V for 2 h. In the next step, the membrane was blocked at room temperature for 60 min in TBS (Tris-buffered saline) containing Tween-20 (TBST) and 5% non-fat milk, followed by incubation at 37°C for 120 min with goat anti-ADRA2A (ab45871, 1:5,000 dilution, Abcam) or anti-β-actin primary antibodies (4970s, 1:9,000 dilution, Cell Signaling Technologies). Subsequently, the membrane was washed and incubated at room temperature for 2 h with horseradish peroxidase (HRP)-conjugated anti-goat secondary antibodies (ab6721, 1:13,000 dilution, Abcam). A Western Lightning Enhanced Chemiluminescence solution (Perkin Elmer) was used to detect the protein signals, which were acquired and analyzed using ImageJ software (NIH) to obtain the density of each protein band. All experiments were performed 3 times.

Statistical analysis. The data were analyzed by non-parametric tests and the significance of different variables was compared using one-way ANOVA, followed by Dunnett's test as a post hoc test. All continuous variables were presented as medians and a P-value of <0.05 was considered to indicate a statistically significant difference. All statistical analyses were carried out using SPSS 18.0 software (SPSS, Inc.).

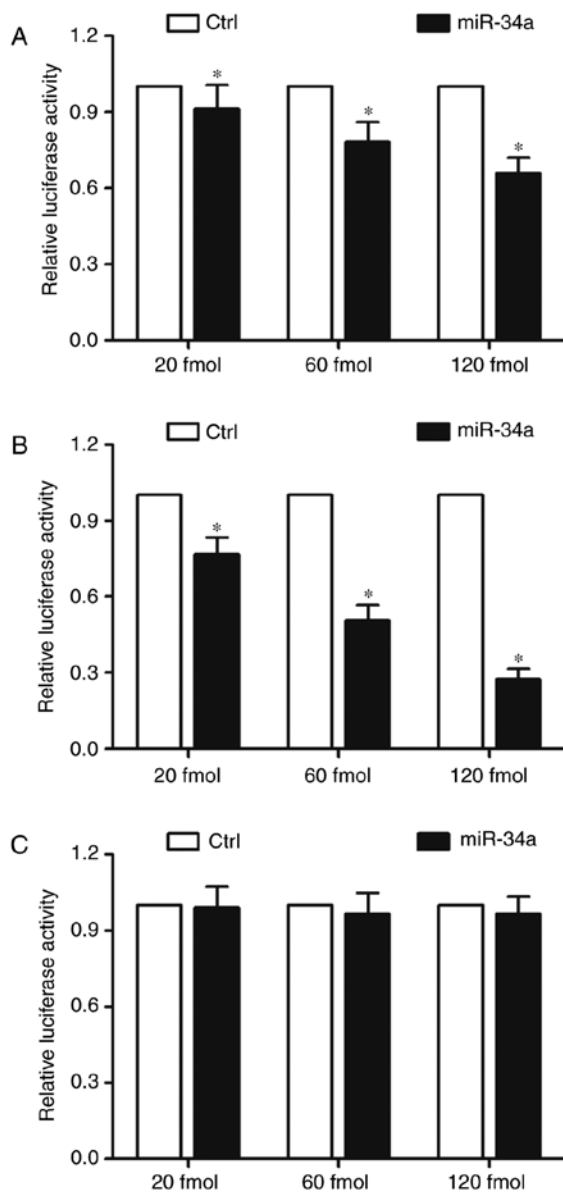


Figure 2. rs3750625 allele influences miR-34a binding in HCN-1A cells. (A) miR-34a mimic reduced the luciferase activity of major ADRA2A 3'UTR in a dose-dependent manner compared with the control. (B) miR-34a mimic dose-dependently inhibited the luciferase activity of minor ADRA2A 3'UTR compared with the control. (C) miR-34a mimic had no effect on the luciferase activity of mutant ADRA2A 3'UTR compared with the controls. *P<0.05 compared with the control. ADRA2A, adrenoceptor alpha 2A.

Results

rs3750625 is located in the seed region of ADRA2A containing a putative binding site for miR-34a. According to the results of in silico analysis by the utilization of miRdSNP (<http://mirdsnp.ccr.buffalo.edu/>), rs3750625 was located in the seed region of ADRA2A containing a putative binding site for miR-34a. In addition, A allele, the minor allele in rs3750625 polymorphism, could increase the binding affinity between ADRA2A and miR-34a by creating an A-U base pair. Furthermore, the 'seed sequence' in the 3'UTR of ADRA2A was highly conserved among different species (Fig. 1A), indicating that such sequence may play a very important role. As shown in Fig. 1B, the binding of miR-34a to ADRA2A was successful when the major allele

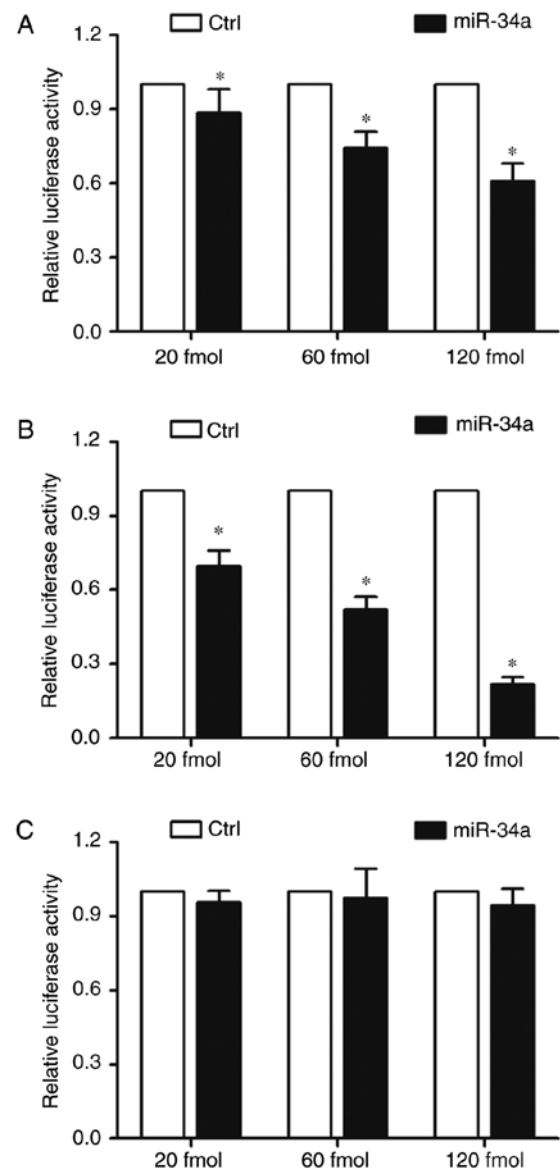


Figure 3. rs3750625 allele influences miR-34a binding in U251 cells. (A) miR-34a mimic reduced the luciferase activity of the major ADRA2A 3'UTR in a dose-dependent manner compared with the control. (B) miR-34a mimic dose-dependently inhibited the luciferase activity of the minor ADRA2A 3'UTR compared with the control. (C) miR-34a mimic had no effect on the luciferase activity of mutant ADRA2A 3'UTR compared with the control. *P<0.05 compared with the control. ADRA2A, adrenoceptor alpha 2A.

of ADRA2A was present; however, the mutation in ADRA2A 3'UTR hindered such binding.

rs3750625 allele affects miR-34a binding. According to our results from an online search upon miRDB, miR-34a could directly target ADRA2A 3'UTR (Fig. 1B). To further confirm the interaction between miR-34a and ADRA2A, the major, minor or mutant 3'UTR of ADRA2A was inserted into a pGL3 vector immediately downstream of the luciferase reporter gene (Fig. 1B), followed by the co-transfection of HCN-1A and U251 cells with these luciferase reporter constructs and various concentrations of the miR-34a mimics (20 fmol, 60 fmol and 120 fmol). As shown in Figs. 2A and 3A, transfection with miR-34a mimics reduced the luciferase activity

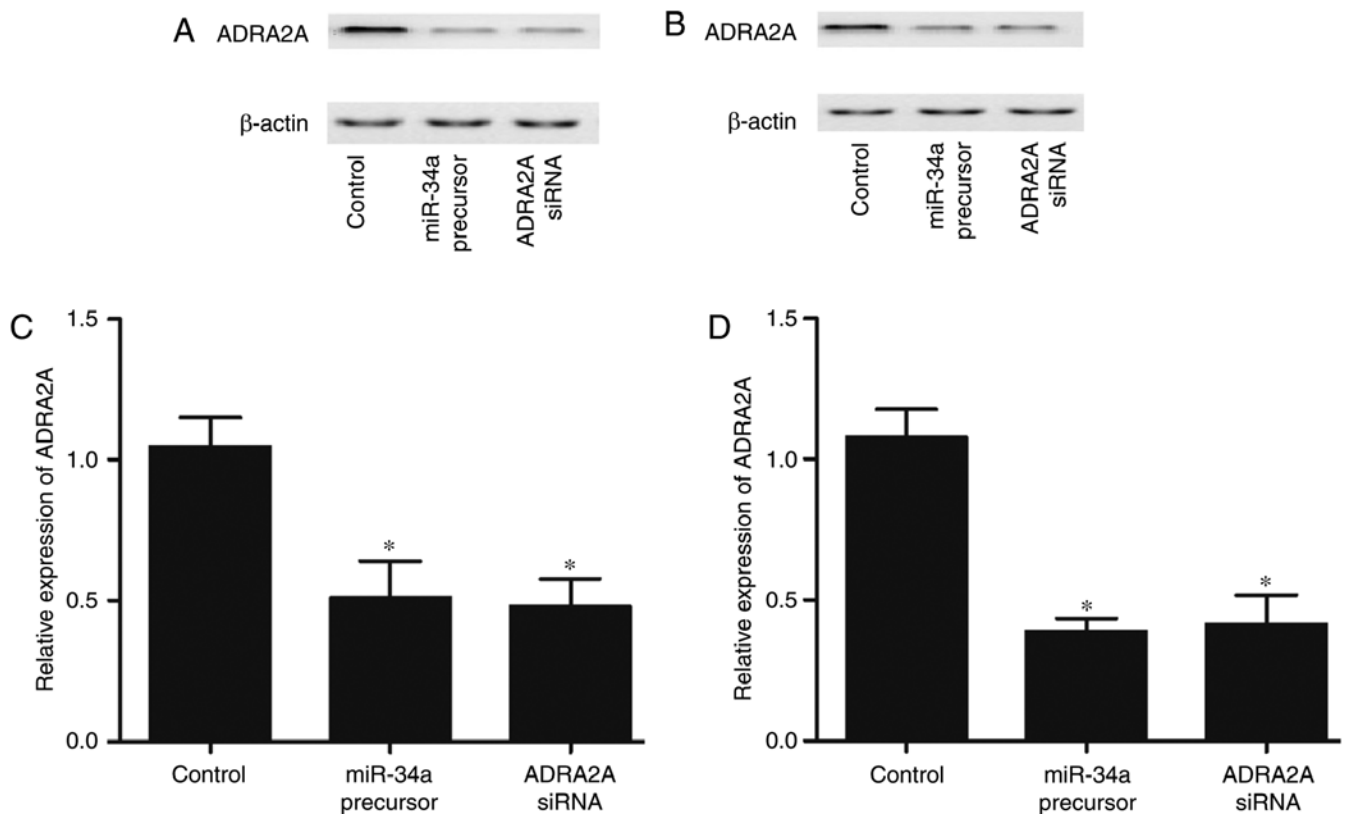


Figure 4. miR-34a suppresses ADRA2A expression. (A) ADRA2A protein level in HCN-1A cells transfected with miR-34a mimic was much lower than that of the control. (B) U251 cells transfected with miR-34a mimic exhibited a lower protein level of ADRA2A compared with the control. (C) ADRA2A mRNA level in HCN-1A cells transfected with miR-34a mimic was much lower than that of the control. (D) U251 cells transfected with miR-34a mimic exhibited a lower mRNA level of ADRA2A compared with the control. * $P < 0.05$ compared with the control. ADRA2A, adrenoceptor alpha 2A.

of the cells transfected with the major ADRA2A 3'UTR, and such a decrease in luciferase activity was dose-dependent. Furthermore, the luciferase activity in the cells transfected with the major ADRA2A 3'UTR decreased in a stepwise manner at an increasing concentration of miR-34a mimics (Figs. 2B and 3B). Notably, when the concentration of miR-34a remained the same, the inhibitory effect of miR-34a on the luciferase activity of the cells transfected with the minor ADRA2A 3'UTR was much stronger than that of the cells transfected with the major ADRA2A 3'UTR. As expected, miR-34a mimics exerted no effect on the luciferase activity of the cells transfected with the mutant ADRA2A 3'UTR (Figs. 2C and 3C), suggesting that the A allele located in rs3750625 increased the binding affinity between ADRA2A and miR-34a, thus reducing the expression of ADRA2A.

miR-34a suppresses ADRA2A expression. To further confirm whether miR-34a inhibits ADRA2A expression, the HCN-1A and U251 cells were transfected with miR-34a precursor or ADRA2A siRNA, as shown in Fig. 4. Following transfection with miR-34a precursor or ADRA2A siRNA, both the HCN-1A (Fig. 4A and C) and U251 (Fig. 4B and D) cells exhibited a decreased protein and mRNA expression of ADRA2A.

miR-34a and ADRA2A are differentially expressed in the different groups. The 38 subjects in this study were divided into 2 groups, i.e., the CC (n=23) and AC (n=15) groups, according to their genotype of rs3750625. Subsequently, RT-qPCR and

western blot analysis were performed to measure the levels of miR-34a, ADRA2A mRNA and ADRA2A protein in these 2 groups. As shown in Fig. 5A, the level of miR-34a in the CC group was similar to that in the AC group; however, the protein and mRNA level of ADRA2A in the CC group was much higher than that in the AC group (Fig. 5B and C).

Determination of sleep quality in different groups. The duration of sleep and the depth of sleep in the 2 groups were continuously monitored and recorded using a Covidien BIS VISTA machine. As shown in Fig. 6, sleep time (Fig. 6A) and sleep efficiency (Fig. 6B) in the CC group were much higher than those in the AC group, indicating that ADRA2A rs3750625 polymorphism was associated with the sleep quality of patients in the ICU.

Mean arterial pressure (MAP), heart rate and respiratory rate in the different groups. Compared to the baseline values, the value of MAP in the AC group was similar to that in the CC group. In fact, the MAP values in both AC and CC groups remained stable from 22:00 to 07:00, measured every 1.5 h (Fig. 7A). In addition, a lower heart rate was observed among patients in the CC group as compared to those in the AC group (Fig. 7B). Furthermore, compared to the baseline values, the subjects in the CC group exhibited a significantly reduced heart rate tendency (Fig. 7B). As shown in Fig. 7C, no obvious difference was observed between the AC and CC groups in terms of respiratory rate.

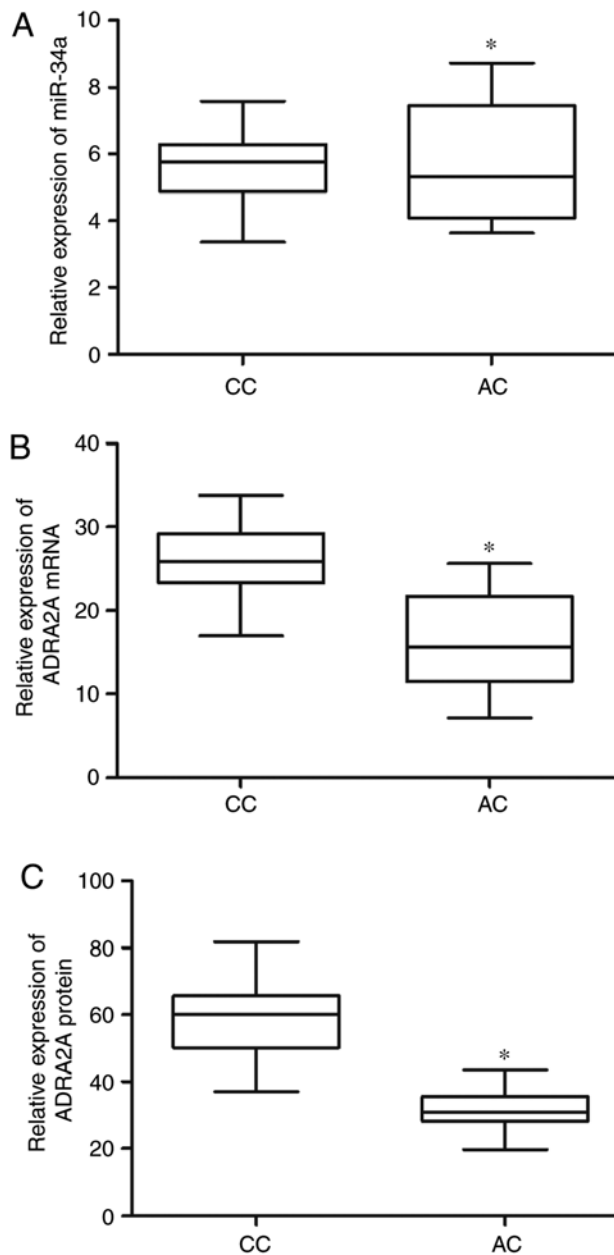


Figure 5. miR-34a and ADRA2A are differentially expressed in the different groups. (A) miR-34a mRNA in AC group exhibited no obvious difference with that in the CC group. (B) ADRA2A mRNA was highly expressed in the CC group compared with the AC group. (C) ADRA2A protein level in the CC group was much higher than that in the AC group. * $P < 0.05$ compared with the control. ADRA2A, adrenoceptor alpha 2A.

Discussion

Poor sleep is very common among patients in the ICU and hence, sleep quality is considered an important aspect of intensive care (25). In fact, patients in the ICU spend approximately 50% of their total sleeping time during the daytime, with a significantly reduced or missing circadian rhythm (26). Furthermore, inadequate sleep may cause extended hospitalization, as well as an extended stay in the ICU. In severe cases, inadequate sleep can trigger long-term cognitive impairment and delirium (27). Therefore, sedation is critical for a successful treatment of patients in the ICU as it can reduce pain, relieve anxiety and decrease stress.

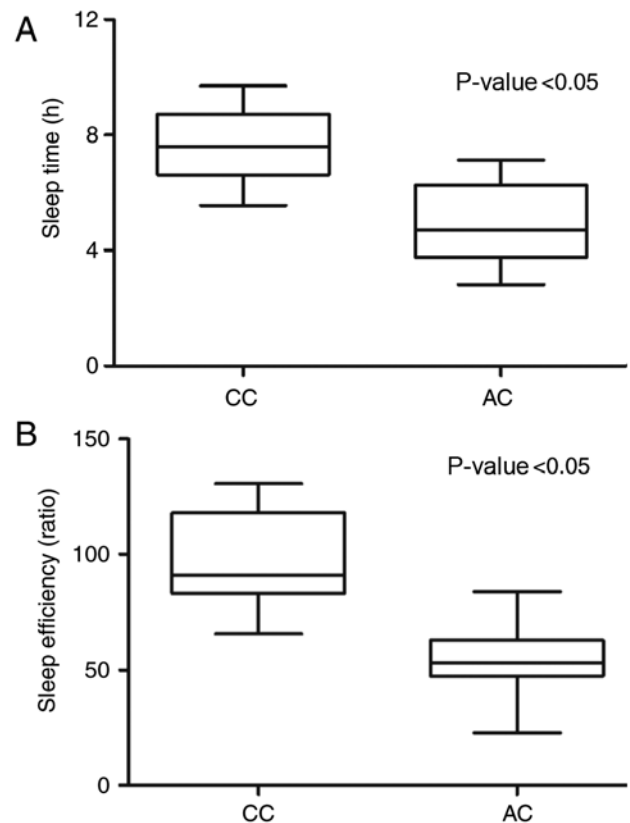


Figure 6. Determination sleep quality between the AC and CC group. (A) Sleep time in the CC group was much higher than that in the AC group. (B) Sleep efficiency in the CC group was much higher than that in the AC group. $P < 0.05$ compared with the CC group.

The adrenergic neurons in the brain are closely related to the regulation of sleep cycles (27). For example, the activation of $\alpha 1$ and β receptors can induce neuronal excitation, while the induction of inhibitory $\alpha 2A$ receptors by agonists, including dexmedetomidine, xylazine or clonidine, can cause hypnosis (28,29). Furthermore, the knockdown or deletion of the *ADRA2A* gene, which encodes the $\alpha 2A$ adrenergic receptor, can reduce the capability of dexmedetomidine to trigger LORR (30). Since the location of *ADRA2A* expression in the brain is highly restricted, it is possible to not only identify which downstream pathways are used by dexmedetomidine to trigger sleep, but also determine which pathways can inhibit sleep, thus allowing us to gain insight into natural sleep circuitry (31,32).

The *ADRA2A* gene is found in chromosome 10q24-q26 and is located in a linkage region to T2DM (33). It was shown previously that *ADRA2A* is involved in the function of β cells (34). Animal models have been used to study the function of miRNAs in the regulation of sleep. For example, sleep deprivation can lead to a significant change in miRNA profiles in rat adipose tissues and brain, and such a change is independent of the plasma level of corticosterone (35). In addition, sleep in experimental animals can be altered by the cortical or intraventricular injection of miRNAs and specific anti-miRNAs into the brain, as suggested by the slow wave activity on electroencephalographic (EEG) (36). The role of miRNAs in the control of sleep quality may be mediated by their ability to regulate the expression of sleep associated

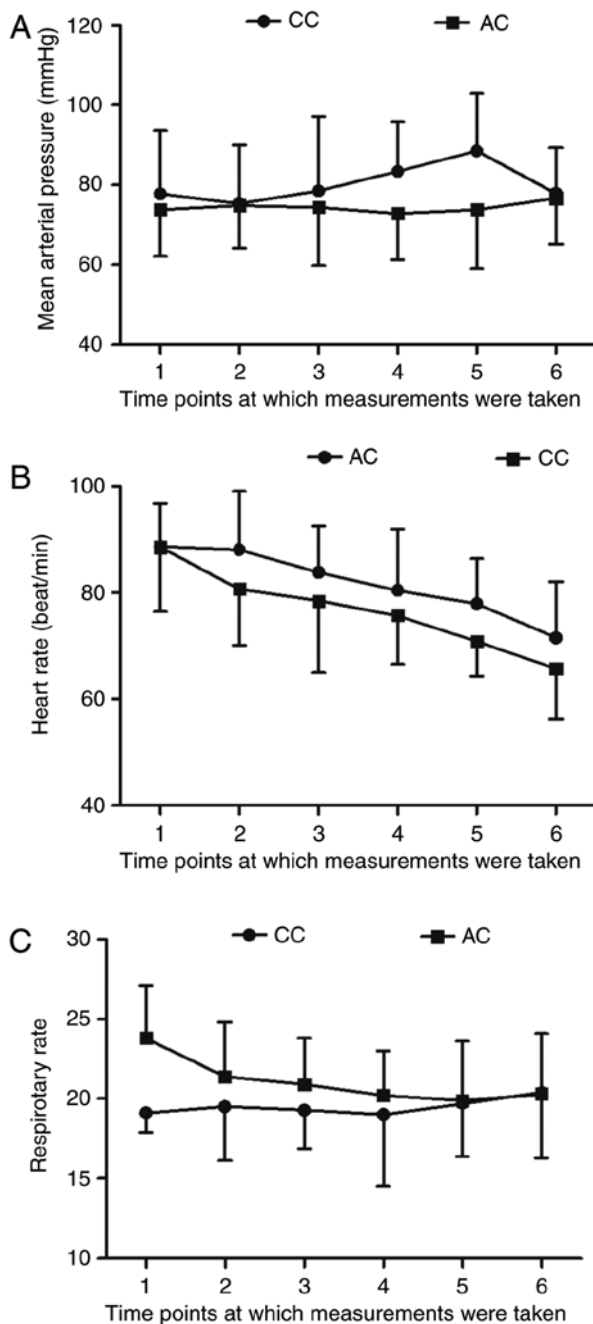


Figure 7. (A) The trend of mean arterial pressure (MAP) between the AC and CC groups. (B) The heart rate trend between the AC and CC groups. (C) The trend of respiratory rate between the AC and CC groups. Note that the x axes in the figure indicate the time points at which measurements were taken. Measurements were taken over a 9-h time period from 22:00-07:00. Measurements were taken every 1.5 h. The time points labeled 1-6 on the x axes indicate the 6 time points at which measurements were taken.

genes. Recently, the full 3'UTR of ADRA2A was cloned into a luciferase reporter plasmid downstream of a Firefly luciferase reporter gene, and the luciferase assay results revealed a potential location for miR-34a binding, which in turn led to the reduction in luciferase activity in a dose-dependent manner (18,37). In this study, we searched an online database and found that miR-34a could directly bind to the 3'UTR of ADRA2A. In addition, a single site or a 'seed sequence' in ADRA2A 3'UTR were mutated in this study to generate minor or mutant ADRA2A 3'UTR, which were then inserted

into pGL3 vectors to generate different luciferase constructs. Furthermore, the above-mentioned luciferase constructs were transfected into the HCN-1A and U251 cells, and the results revealed that miR-34a reduced the luciferase activity of the cells transfected with the major or minor ADRA2A 3'UTR in a dose-dependent manner. As expected, miR-34a had no effect on the luciferase activity of the cells transfected with the mutant ADRA2A 3'UTR.

It was previously suggested that, following sexual assault or MVC-associated trauma, subjects harboring the rs3750625 minor allele may show a 1-point increase (on a scale of 0-10) during acute MSP. This may be caused by a more efficient binding between ADRA2A 3'UTR and miR-34a in the presence of the minor allele. Such results are consistent with the role of miR-34a in the regulation of stress, indicating that the symptoms of stress may be caused by the activation of physiologic systems that also promote miR-34a expression and increase the effect of rs3750625 on stress (18,38). In this study, we recruited 38 patients in the ICU and divided them into 2 groups, i.e., the CC (n=32) and AC (n=6) groups, according to their genotypes of rs3750625. The results revealed that miR-34a expression in the CC group was comparable to that in the AC group, whereas the mRNA and protein level of ADRA2A was significantly higher in the CC group compared to that in the AA group. In addition, we found that the sleep time and sleep efficiency in the CC group were much higher than those in the AC group. Furthermore, we revealed that the MAP value in both the AC and CC groups remained stable from 22:00 to 08:00, and their respiratory rates were also similar. However, a lower heart rate was observed in the patients from the CC group. Donello *et al* previously demonstrated that animals displayed acute hyperalgesia upon sound exposure if ADRA2A was absent (39). It has also been shown that, in individuals harboring the minor allele of rs3750625, an increased level of miR-34a binding, a reduced level of ADRA2A transcripts, and an increased level of acute MSP were observed in those suffering from trauma (18). rs3750625 has been previously shown to play a role in the postprandial gastric volume of obese or overweight European-Americans (22). It has also been demonstrated that rs3750625 is close to or within the seed region of 23 miRNAs, suggesting that rs3750625 may increase the expression of ADRA2A by affecting miRNA binding (18). In a recent study, Linnstaedt *et al* demonstrated that rs3750625 further decreased ADRA2A expression by increasing miR-34a binding affinity, and hence reduced the risk of gestational diabetes mellitus (GDM) by increasing the release of insulin (18).

There are limitations to this study: Firstly, the sample size of this study was relatively small, and further studies with a larger sample size are warranted to confirm the conclusions of this study; secondly, further functional studies are warranted to confirm the conclusions of this study.

Taken together, it can be concluded that the SNP rs3750625 in the 3'UTR of ADRA2A affects the sleep quality of patients in the ICU in by increasing the binding of miR-34a to ADRA2A. In brief, the presence of the rs3750625 minor allele in the 3'UTR of ADRA2A increases the binding between miR-34a and ADRA2A, a direct target of miR-34a. As a result, by further inhibiting the expression of ADRA2A, the presence of the rs3750625 minor allele can affect the sleep quality of patients in the ICU.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

QH and JZ were involved in the conception and design of the study. GC, YH and JL were involved in the literature collection. QH, GC, YD, SZ, XC, QX and LX were involved in data collection. YH, JL, QN and JZ were involved in data analysis and processing. QH, GC and JZ were involved in manuscript preparation. YH, JL, YD, SZ, XC, QX, QN and LX were involved in the final proofreading of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The Human Research Ethics Committees of Dongguan Houjie Hospital approved this research. Written informed consent was obtained from all patients or their first-degree relatives prior to the onset of this research. The research process was in conformity with the latest version of Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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