Integrative analysis of candidate MicroRNAs and gene targets for OSA management using in silico and in-vitro approach

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ABSTRACT

MicroRNAs (miRNAs) have been implicated in the pathogenesis of human diseases including sleep disorders. The aim of this study is to address the involvement of miRNAs (miR-21 and miR-29) in the pathophysiology of obstructive sleep apnea (OSA). In this study we have done integrated analysis of miRNAs with their potential gene targets as a strategy for management of OSA.

Methods: miRNA expression levels were quantified in healthy control group and obese vs. Non-obese OSA subjects by Quantitative real-time PCR. In-silico analysis of interplay of miRNAs with potential gene targets was done using Schrödinger Release 2023-1.

Results: The real time expression analysis revealed a differential expression pattern in miRNAs indicating downregulation of miR-21 in obese OSA while miR-29 showed upregulation as compared to non-obese OSA and healthy subjects with p values of ≤ 0.01 and < 0.0001 respectively. A trend was observed where target genes TGFBR2, NAMPT, and NPPB were significantly increased with p-value of ≤ 0.0001 and TGFBR3 and INSIG2 showed decreasing trend with p-value of ≤ 0.0001 between obese and non-obese OSA respectively. MD simulation analysis provided valuable information regarding the stability, flexibility, compactness and solvent exposure of the complexes over time.

Conclusion: miR-21 and miR-29 possesses differential expressions in obese OSA subject and exihbits strong molecular interactions with potential target genes, such as TGFBR2, NPPB, NAMPT and INSIG2. Identifying the miRNAs, genes and pathways associated with OSA can help to expand our understanding of the risk factors for the disease as well as provide new avenues for potential treatment.

1. Introduction

Envision a suffocating grip of darkness around our neck leaving us choking while we lie in the embrace of sleep. This is a devastating situation where we lie partially paralyzed in fear due to the complete influence of over relaxation of throat muscles resulting in collapsed soft tissues of our throat which facilitates in partial or complete blockage of upper respiratory passage, resulting to a haunting breathlessness. This complete condition is known as Obstructive Sleep Apnea (OSA). OSA may lead to serious health problems like cardiovascular disorders, depression, hypertension, decreased levels of oxygen, obesity and many more psychiatric disorders.[1]

Sleep deprivation and OSA syndrome severely escalate the levels of inflammatory biomarkers such as CRP, TNF-α, IL-6, IL-8, CAMs *etc*.[2–8] However, there are certain limitations to protein and cytokine biomarkers such as low sensitivity and specificity during early diagnosis and they are usually observed to be elevated in any kind of infections or diseases. The fact that OSA mortality is going steeply high due to its association with metabolic complications.[9–11] Therefore, to optimize the treatment strategy,it is crucial to detect the early signs of OSA complications.[12] This calls for exploration of a different, more specific dimension of biomarkers- MicroRNAs (miRNAs).

Several studies have focussed on miRNA investigations and their utility as independent diagnostic and prognostic markers of OSA and associated altercations.[13–19] However, no study till date has emphasized the role and molecular mechanisms behind miRNA regulations in obesity associated OSA.

In our previously published study, we identified two miRNA signatures related to OSA, miR-27 and let-7. These miRNAs are involved in the TGF-β signalling pathway and we also speculated their involvement in the pathophysiology of OSA.[20] However, considering the Indian subcontinent, there is very less evidence that highlights the biomarkers in OSA. Also, reports pertaining to miRNA profiling in the Indian sub-population of OSA are not known. Our study focuses on the role of miR-21 and miR-29 as potential candidate for OSA study as these two miRNAs having well highlighted roles in OSA associated disorders such as CVD and obesity, but their role in OSA is still elusive as most exquisite regulatory molecular signature and therapeutic relevance's of their related gene targets. The data selection of miRNA and their target genes were done to evaluate them as early markers of OSA associated with obesity.

OSA diagnosis in complex metabolic inter-related disorders is mostly inferential leading to detrimental effects. The exploration of molecular signature such as miRNAs, genes and proteins expressed in clinical conditions to generate a pathophysiological pattern in OSA is intriguing for therapeutic relevance. The identification of these specific mRNA target sites for a therapeutic remediation aided via the mechanism of miRNA-mediated gene silencing (RISC) which refers to repression of the targeted translational process, is facilitated by human Argonaute protein (AGO). The analysis of these AGO-miRNA complexes were done by performing Molecular Dynamics (MD) simulation of the crystal structure of human AGO2 protein complex with miR-21-NPPB, mir-21-TGFBR2 and miR-29-INSIG2, mir-29-NAMPT and mir-29-TGFBR3 duplexes.

Here in this study we (a) attempted to understand if obesity plays a role in OSA in terms of two potential miRNAs expression (miR-21 and miR-29) which are known to have a role in metabolic disorders associated with obesity and various diseases (b) identification of a set of potential targets through in-silico analysis in OSA model and their validation in OSA patients. We hypothesize that understanding these differential expressions of miR-21, miR-29 and their potential target genes associated with lipid metabolism could lead to the identification of potential biomarkers for diagnosing obesity-related OSA and developing targeted therapies aimed at restoring normal miRNA levels or inhibiting their dysregulated targets.

2. Materials and methods

2.1. Selection of miRNAs

MiR-21 and MiR-29 were selected in our study as as these two miRNAs are having well highlighted roles in obesity and obesity associated disorders like CVD etc.[21–23] The miR-29 family dysregulation is associated with obesity, insulin resistance, and type 2 diabetes.[24–27] However, an unanswered question arises, whether they have prominent roles in regulating the pathophysiology of OSA or not. Interestingly, these miRNAs (miR-21 and miR-29) surfaced through the NGS study by Li et al. in atherosclerosis associated OSA subjects.[13] This is our initiating point in our study design for understanding the role of miRNAs in our patient cohorts and further in depth analyses of their corresponding gene targets.

2.2. Study participants

This study is a continuation of our previously conducted investigations from the patients that were enrolled for the study.[28] For this prospective study, a total of 160 subjects were recruited in the Out-Patient Department of Pulmonary Diseases and critical care, Safdarjung Hospital and All India Institute of Medical Sciences, New Delhi
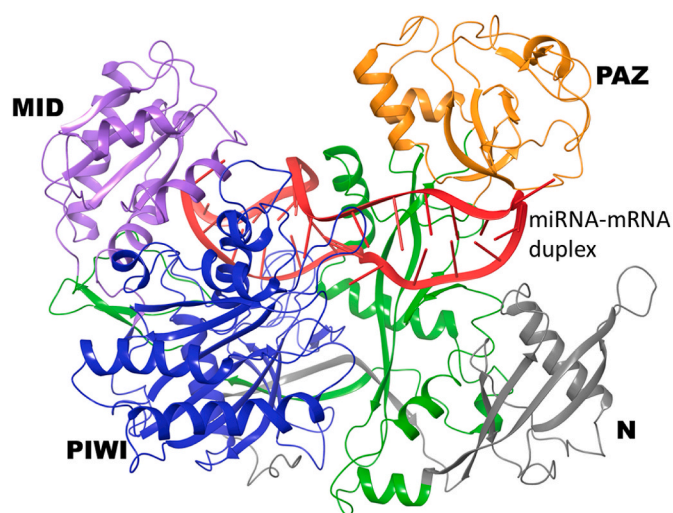

Fig. 1. Structure of human Ago2 protein with miRNA-mRNA duplex. The Ago protein is shown as cartoon with each of its domains in different colour. The miRNA-mRNA duplex is shown in red.

who underwent screening procedures. Of these, subjects with cardiovascular and metabolic disorders such as diabetes (n = 70) were excluded from the study. The remaining 90 subjects were divided into 3 groups based on their BMI-(i) obese (BMI = 33.56 ± 3.93),subjects with polysomnography-proven OSA (n = 35), (ii) non-obese (BMI = 26.31 ± 2.31) (with polysomnography-proven OSA (n = 35) and (iii) control subjects (BMI = 23.65 ± 2.32). who were exclusive of any disorders (n = 20). The subjects recruited were between 35 and 50 years of age of either gender.

2.3. Isolation of RNA and real time PCR

As elaborated by our team in our previous investigation reports, miR-21 and miR-29 expression analysis was performed using Power-Up SYBR Green Master-mix (Applied Biosystems, Austin, TX) based real time PCR. cDNA samples were run in triplicates and normalized with RNU48 and non-template controls. The fold change was calculated based on the obtained C_t values using the $2^{-\Delta\Delta Ct}$ method.

2.4. Statistical analysis

Statistical analysis was performed using SPSS Statistics Software (Version 22.0). Student's t-test was employed to generate p-values. Spearman's correlation test was employed to test a correlation between two variables. $p < 0.05$ was considered as statistically significant.

2.5. MD simulations

MD simulation techniques have been employed to explore the dynamic behaviour of the gene structures at the atomic level. Crystal structure of the Human Arganaute2 (AGO2) (PDB ID: 6N4O) bound to the guide-mRNA was obtained from the Protein Data Bank (PDB) (Fig. 1). The co-ordinates of guide-mRNA and miRNA were modelled manually (Fig. 2). Target protein preparation was done using Protein Preparation Wizard module of Schrödinger Release 2023-1, followed by optimization using the OPLS4 force field. This process involved adding hydrogen atoms, assigning bond orders and charge states, optimizing hydrogen bonds and resolving atomic clashes. Molecular dynamics (MD) simulations were performed in Desmond (Schrödinger Release 2023-1). Protein–ligand complexes were solvated with the TIP3P water model and sodium ions (Na+) were introduced to neutralize the system. The system underwent energy minimization for 100 ps before initiating the MD simulations. Each complex was then subjected to a 200-ns
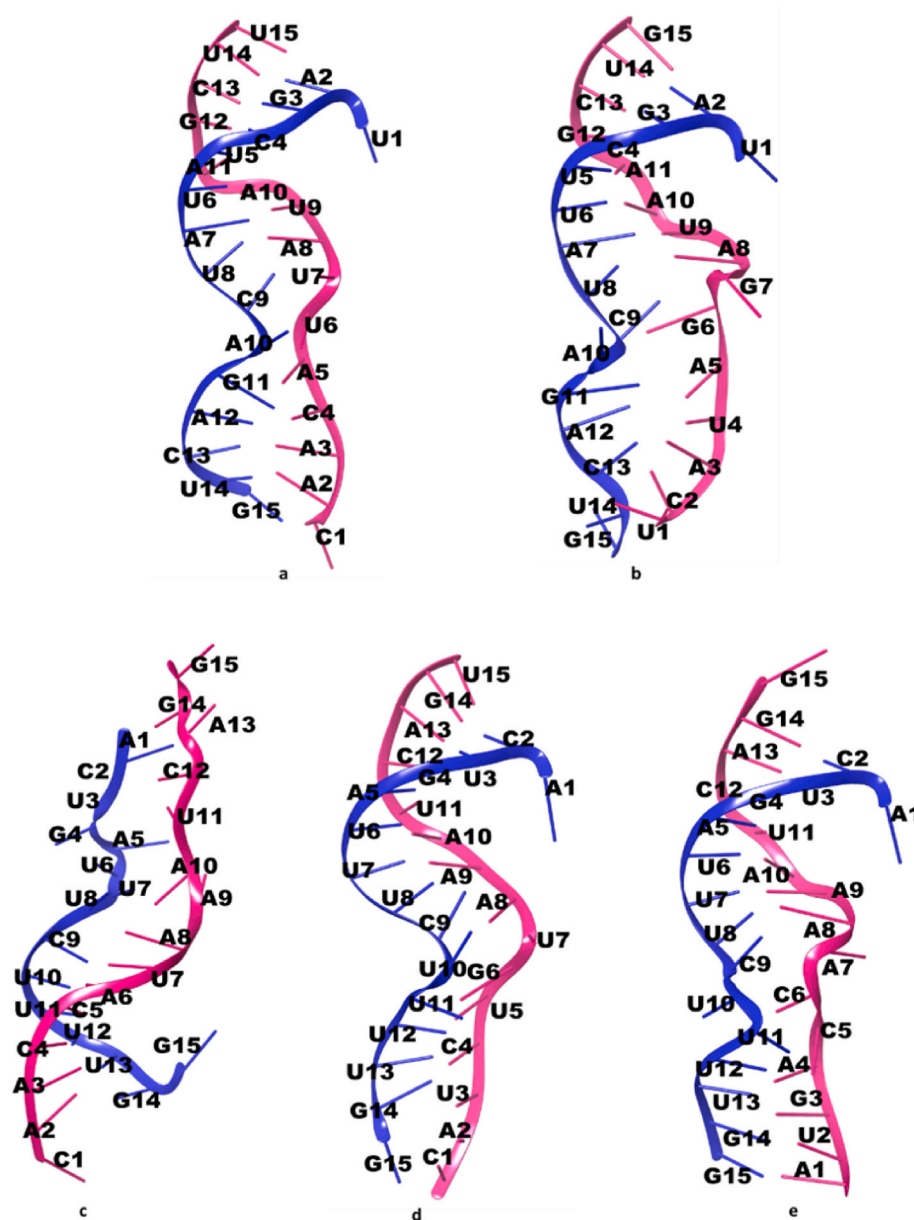
Fig. 2. Structure of 15-nt guide (miRNA) (blue) and target (mRNA) (pink) duplex of (a) miR21-NPPB (b) miR21-TGFBR2 (c) miR29-NAMPT (d) miR29-INSIG2 and (e) miR29-TGFBR3.
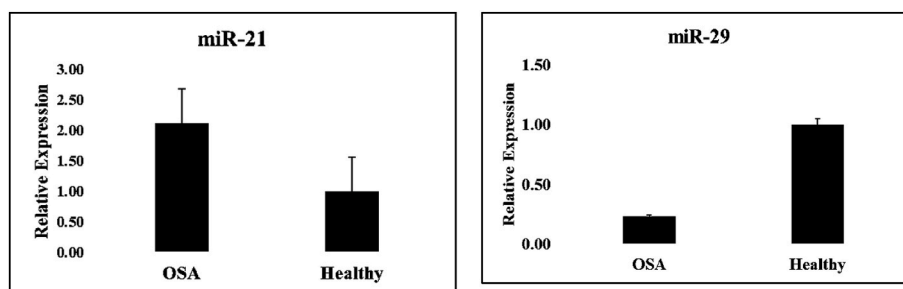

Fig. 3. Relative expression levels of miRNAs in OSA and Control groups. miR-21 was observed to be upregulated and miR-29 was found to be downregulated in OSA (n = 100) compared to the healthy controls (n = 60). The data is representative of 3 consecutive experiments. ***P < 0.001.

production run at 310.15 K and 1.01325 bar pressure using the Nose-Hoover method, with trajectory data recorded every 100 ps to produce 2000 frames in the NPT ensemble. The stability of the model and structural changes in the resulting trajectories were analyzed using parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg).
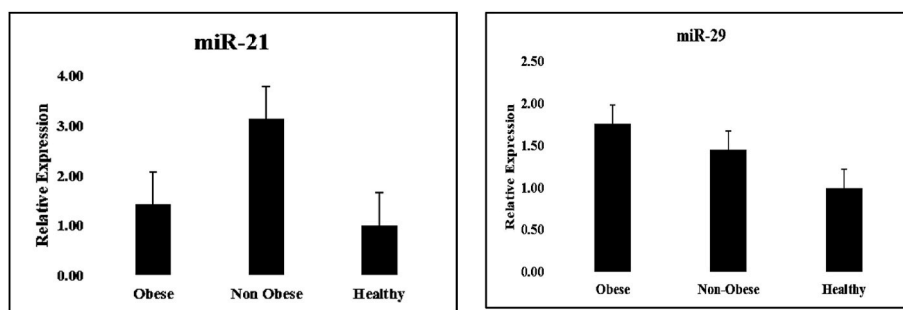
Fig. 4. Association of miRNA expression with obesity. Quantitative PCR data showing relative miRNA expression. The data is representative of 3 consecutive experiments. ***P < 0.0001.
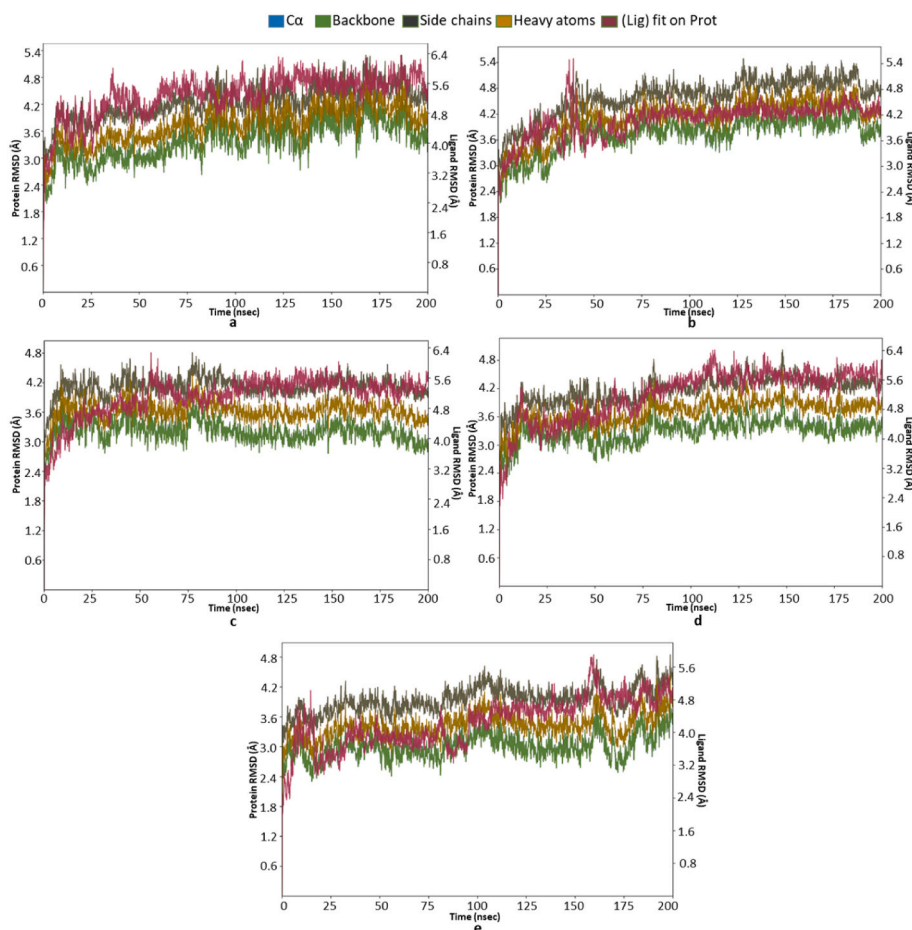

Fig. 5. Structure analysis of MD simulation trajectories obtained for Arganaute protein (AGO) (PDB ID: 6N40) (a) The RMSD plot of miR21-NPPB duplex (b) RMSD plot of miRNA-miR21-TGFBR2 duplex (c) RMSD plot of miRNA-miR29-INSIG2 duplex (d) RMSD plot of miR29-NAMPT duplex (e) RMSD plot of miR29-TGFBR3 duplex. A stable complex is formed without any significant conformational changes in protein structure during 200ns simulation.

3. Results

3.1. Expression profiling of miRNAs in OSA vs healthy

We performed the profiling of miR-21 and miR-29 using quantitative PCR, wherein a differential expression was observed in OSA subjects compared to healthy controls as depicted in Fig. 3. Real time analysis revealed a significant increase in the expression level of miR-21 in OSA patients as compared to the controls (**p < 0.01**), and significant decrease in the expression levels of miR-29 (**p < 0.001**) in OSA.

3.2. Expression profiling of miRNAs in obesity associated OSA

This section pertains to the impact of obesity on miRNA profiles in OSA patients (Fig. 4). Interestingly, our observations highlight a significant increase in the relative expression of miR-21 in non-obese patients (3.14 fold) than the obese OSA (1.42 fold) and healthy subjects (**p = 0.01**). On the contrary, miR-29 levels were elevated in the obese OSA subjects (1.77 fold) compared to the non-obese and controls (**p < 0.0001**).
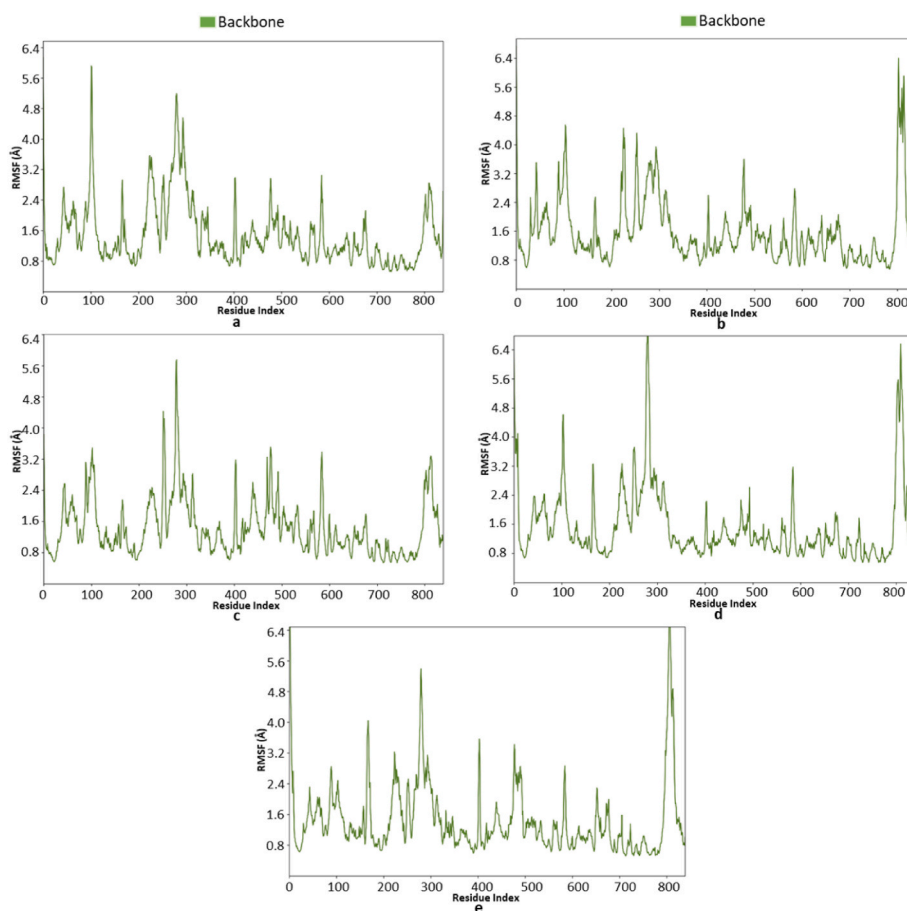
Fig. 6. Backbone RMSF plot of MD simulation trajectories obtained for Arganaute protein (AGO) (PDB ID: 6N40) plotted against residue numbers (a) The RMSF plot of miRNA-miR21-NPPB duplex (b) RMSF plot of miRNA-miR21-TGFBR2 duplex (c) RMSF plot of miRNA-miR29-INSIG2 duplex (d) RMSF plot of miRNA-miR29-NAMPT duplex (e) RMSF plot of miRNA-miR29-TGFBR3 duplex. A stable complex is formed without any significant conformational changes in protein structure during 200ns simulation.

3.3. Identification of target genes

To investigate the molecular mechanisms behind the role of these miRNAs in OSA, it was essential to identify potential target genes. Screening and identification of target genes was performed using web based bioinformatic tools and databases such as miRDB and TargetScan. Potential genes like TGFBR2, TGFBR3, B-type natriuretic peptide (NPPB), insulin induced gene 2 (INSIG2) and nicotinamide phosphoribosyltransferase (NAMPT) were selected for further analysis due to their elaborated roles in obesity, CVD and diabetes, however, their role in OSA is still elusive.

3.4. MD simulation

MD simulation was employed to assess various parameters of the miRNA-mRNA complexes. Specifically, we analyzed root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) were analyzed. These metrics provide valuable information regarding the stability, flexibility, compactness and solvent exposure of the complexes over time. Moreover, the outcomes of the in silico analysis not only corroborate the existing literature findings but also offer supplementary insights, including the identification of target genes and their stability. It is noteworthy that a recent study by Shukla et al. adapted a similar approach and validated the above method.[29]

In order to uncover the realistic interaction of miR-21 against B-type natriuretic peptide (NPPB) and TGFBR2 and miR-29 against insulin induced gene 2 (INSIG2), TGFBR3 and nicotinamide

phosphoribosyltransferase (NAMPT) with AGO2 protein, we performed MD-simulation for 200ns. We examined the RMSD of the Ago protein and miRNA-mRNA duplexes (Fig. 5). The observed deviations in the RMSD values indicated that the complexes were stable throughout the 200ns simulations. The RMSD plot revealed that miR21-NPPB and miR21-TGFBR2 duplexes have reached equilibration after 50ns (Fig. 5a and b). For miR21-NPPB, miR-21-TGFBR2, miR29-TGFBR3, miR29-INSIG2 and miR29-NAMPT the average RMSD is ~2.0–4.0 Å which states that all the complexes are stabilized during the simulation (Fig. 5c, d, 5e).

The RMSF value of the miRNA-mRNA duplex fit on protein backbone over the entire simulation was plotted against residue numbers which is shown in Fig. 6. The observed deviation in the RMSF values provide insights into the flexibility and dynamics of the miRNA-mRNA duplex associated with AGO2 protein. The RMSF of miR21-NPPB is stable around 1.6–2.4 Å. It can be clearly seen in Fig. 6a that the RMSF value for protein backbone amino acids shows large deviations in the N and PAZ domain (~5.0–6.0 Å) in comparison to other protein domains. Whereas in miR21-TGFBR2 complex the RMSF value shows moderate to large deviation in PAZ and PIWI domain respectively (~4.5 and 6.4 Å) (Fig. 6b). This shows that miR21-NPPB duplex is more stable as compared to miR21-TGFBR2. For miR29-INSIG2, the RMSF value shows less deviation in the protein domains as compared to miR29-TGFBR3 and miR29-NAMPT where it can be evidently seen that there are large deviations in PAZ and PIWI domain of the protein (Fig. 6c, d and 6e).

To understand the compactness of the complexes we analyzed Rg (Radius of gyration) as shown is Fig. 7. The analysis revealed that the Rg
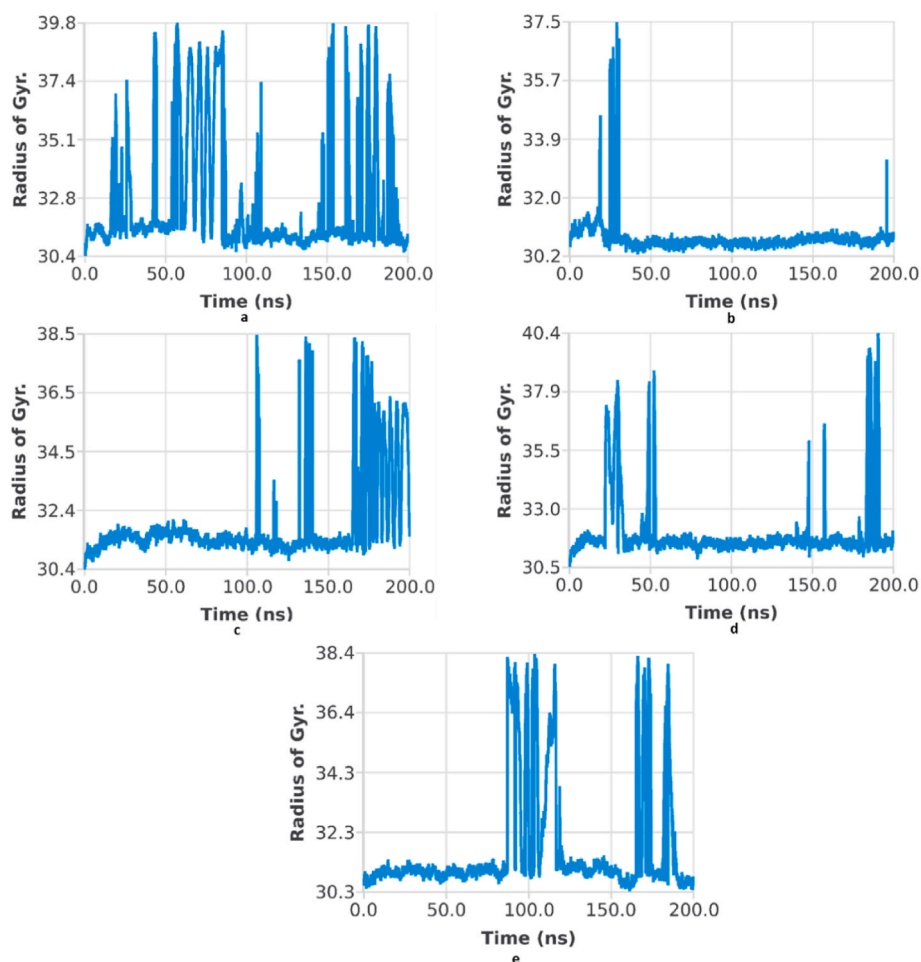
Fig. 7. Rg plots of all the five duplexes obtained for Arganaute protein (AGO) (PDB ID: 6N40) plotted against time (a) The Rg plot of miRNA-miR21-NPPB duplex (b) Rg plot of miRNA-miR21-TGFBR2 duplex (c) Rg plot of miRNA-miR29-INSIG2 duplex (d) Rg plot of miRNA-miR29-NAMPT duplex (e) Rg plot of miRNA-miR29-TGFBR3 duplex.

value of the miR21-NPPB complex was found to be lightly stretched in a range of 39.8–30.4 Å throughout the MD simulation frame of 200ns (Fig. 7a). Rg of miR21-TGFBR2 was found to be in the range of 37.5–30.2 Å (Fig. 7b). This indicates that miR21-NPPB is more compact which is in accordance with RMSF plot. On the other hand the compactness of miR29-NAMPT (40.4–30.5) is more as compared to miR29-INSIG2 (38.5–30.4 Å) and miR29-TGFBR3 complex (38.4–30.3 Å). This suggests that miR29-NAMPT duplex is tightly packed with the AGO2 protein.

3.5. Expression profiling of miRNA regulated targets in OSA

Real time PCR based analysis of the target genes suggested differential expression patterns in OSA compared to healthy participants as depicted in Fig. 8. A decline in the expression profiles were observed in TGFBR2 (~2.7-fold, **p<0.0001**), and NPPB (2.5-fold, **p<0.0001**) whereas elevated expression in NAMPT (3.5-fold, **p<0.0001**) and INSIG2 (4-fold, **p<0.0001**) was observed in OSA subjects. Not much significant change was observed for TGFBR3 (**p=0.05**).

3.6. Association of target gene expression with obesity

Similar to the analysis done for the selected miRNAs, we evaluated the impact of obesity on the target genes in OSA too. A trend of increased expression in the obese subjects compared to the non-obese subjects was observed in TGFBR2 (6-fold, **p<0.0001**), NAMPT (1.8-fold, **p<0.0001**)

and NPPB (5-fold, **p<0.0001**) whereas TGFBR3 had decreased expressions in obese OSA subjects (2-fold, **p<0.0001**). INSIG2 also showed a decreased trend in its expression (5-fold, **p<0.0001**) as depicted in Fig. 9.

4. Discussion

MicroRNAs (miRNAs) therapeutic potential have been investigated thoroughly in most of the diseases ranging from obesity to cancer. However, their role as biomarkers when considered in OSA is still evolving. The considered investigation focuses on two specific miRNA signatures which are (miR-21 and miR-29) in the account of middle aged Indian OSA influenced individuals. We investigated the expression of miR-21 and miR-29 from 70 North Indian OSA patient samples using real-time PCR. Our study revealed significant down-regulation in miR-21 expression level in obese OSA patients as compared to non-obese OSA and healthy non-OSA cohort. Similarly, a study involving treatment-naive severe OSA patients found that levels of miR-21–5p were significantly lower compared to matched subjects with primary snoring.[30,31] This downregulation correlates with increased expression of pro-inflammatory cytokines such as TNF-α, indicating a potential role for miR-21 in modulating inflammation associated with intermittent hypoxia—a hallmark of OSA,[32] indicating miR-21 may be a viable therapeutic target for managing inflammation in OSA patients.

In contrast we have found significant elevation in miR-29 level in obesity induced OSA subjects as compared to non-obesed OSA and
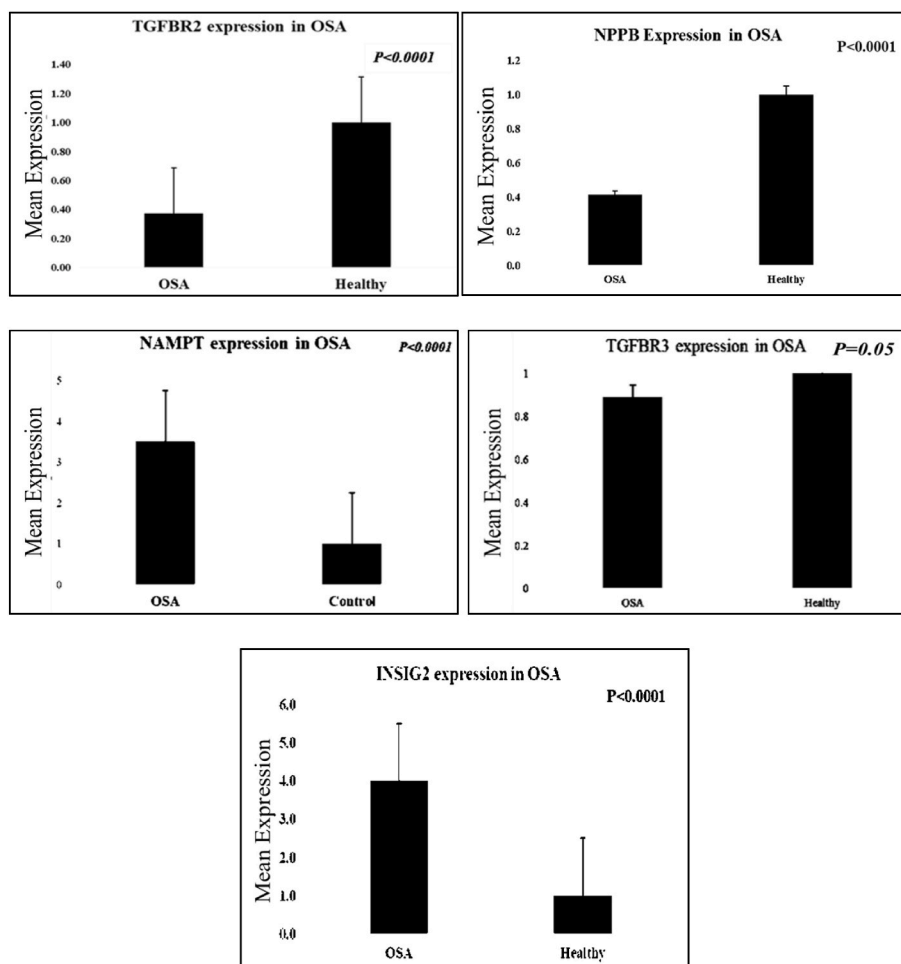
Fig. 8. Expression profile of target genes in OSA. The expression profiles of target genes in OSA as compared to the healthy subjects. P < 0.05 was taken as statistically significant.

healthy subjects. The upregulation of miR-29 could be linked to its involvement in metabolic pathways that are disrupted by sleep apnea. There is limited literature on the involvement of miR-29 in obesity and OSA. Elevated levels of these microRNAs have been observed in multiple tissues, including adipose tissue, liver, and pancreatic β cells. However, the expression of miR-29 is decreased in early stages of obesity, suggesting a complex role that evolves with disease progression.[24–26] The regulatory role of various miRNAs including miR-21 and miR-29 in obesity and obesity related disorders are elaborated in a recent review by Sujay Paul et al., 2023.[27] The altered levels of both miR-21 and miR-29 provides a basis for their use as biomarkers in clinical settings.

Furthermore, identification of molecular targets to these miRNAs such as TGFBR2, TGFBR3, NPPB, INSIG2 and NAMPT by the MD Simulations with the respective miRNAs revealed that these miRNAs can be considered as potential candidates in treatment of OSA as they interact substantially with their target genes of OSA linked comorbidities.

The study revealed down-regulation of TGFBR2 in OSA, while minimal change in TGFBR3 mRNA expression observed between the two cohorts. In addition, our studies demonstrated down-regulation of TGFBR3 in OSA patients having comorbidity like obesity. These findings are according to previous reports on visceral obesity, where TGFBR3 expression was significantly decreased correlated to insulin resistance and higher risk of type 2 diabetes and metabolic diseases.[33,34] TGFBR2 and TGFBR3 has been studied in obesity and associated cardiovascular and metabolic disorders suggesting that elevated levels of TGFBR2 due to high glucose levels is a contributing factor in diabetic nephropathy. Similar trend in elevation in TGFBR2 was found in obese OSA subjects in

our study. While specific studies directly linking TGFBR2 in obstructive sleep apnea (OSA) are limited, the role of TGF-β signaling pathways in inflammation and tissue remodeling suggests potential relevance in OSA as characterized by intermittent hypoxia and inflammation. Understanding these mechanisms could lead to novel therapeutic strategies targeting TGF-β signaling pathways in treatment and management of OSA-related complications.

A literature cites overexpression of natriuretic peptide (NPPB) lowers the blood pressure thereby protecting against hypertension and obesity.[35] This study corroborates with our findings where a down-regulation in the expression of NPPB in OSA subjects was observed. However, the expression increased in the obese cohorts compared to the non-obese cohorts. Variants in the regulatory regions of the NPPB gene have been associated with hypertension and heart failure, which are common complications in OSA patients. This highlights the role of genetic factors to modulate NPPB expression and its subsequent effects on cardiovascular outcomes.[36] The differential expression of NPPB and related genes in the context of OSA could serve as potential biomarkers for diagnosing and monitoring the severity of the condition.

Similarly, Nicotinamide phosphoribosyltransferase (NAMPT) is a crucial target in obesity. Inhibition of NAMPT expression has been seen to prevent obesity in mice.[37] In patients with OSAHS, serum levels of NAMPT are markedly elevated compared to healthy individuals, suggesting that hypoxia associated with OSA may stimulate NAMPT expression.[38] In our study also there was a significant upregulation of NAMPT was observed in the OSA cohort as well as in the obese OSA subjects, suggesting that the interplay between NAMPT and obstructive
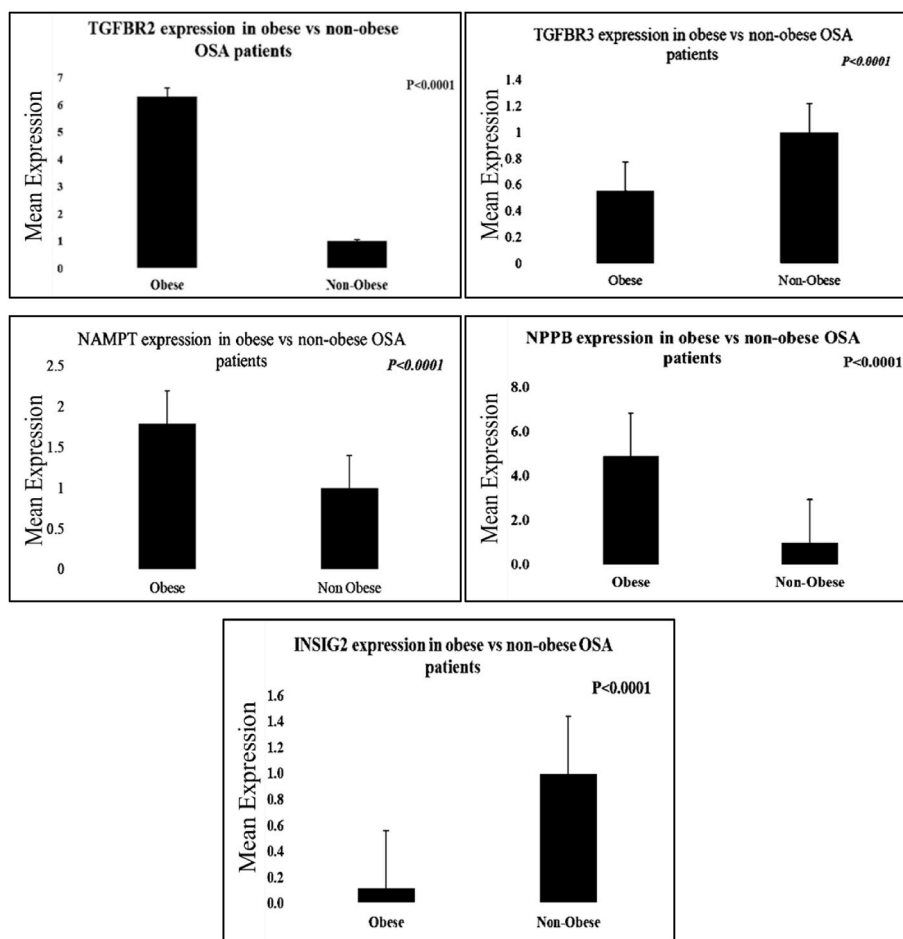
Fig. 9. Association of target gene expression with obesity. Quantitative PCR data showing target gene expression in obese vs. non-obese OSA subjects. The data is representative of 5 consecutive experiments. P-values were generated using Student's t-test. P < 0.05 was taken as statistically significant.

Table 1

Differential expression of target genes in obese and non-obese OSA.

miRNA	Target Gene	Obese OSA	Non-Obese OSA
miR-21	TGFBR2	↑	↓
	NPPB	↑	↓
miR-29	NAMPT	↑	↓
	INSIG2	↓	↑
	TGFBR3	↓	↑

sleep apnea highlights the enzyme's potential role as both a biomarker and a therapeutic target in managing cardiovascular complications associated with OSA.

Our further investigation revealed another important player, insulin-induced gene 2 (INSIG2), association with obesity. Evidence suggests a functional role of INSIG2 in obesity, due to its involvement in cholesterol and fatty acid synthesis feedback inhibition.[39] Evidence also reveals that polymorphism in INSIG2 has been identified as a good candidate gene in obesity.[40,41] We reported an upregulation in INSIG2 in the OSA cohort compared to the healthy cohort while decreased expression levels in obese subjects of OSA compared to the non-obese subjects. Table 1 depicts the differential expression of target genes in obese and non-obese OSA. The down-regulation of **INSIG2** in the context of **obesity** and **obstructive sleep apnea (OSA)** has been a subject of investigation due to its implications in metabolic processes and adipocyte function. While specific studies directly linking INSIG2 down-regulation to OSA are limited, the relationship between obesity and OSA is well-established. Given that obesity is a major risk factor for

OSA, the metabolic dysregulation associated with decreased INSIG2 function could theoretically contribute to the severity of OSA symptoms. Its genetic variants may influence susceptibility to obesity and related metabolic disorders. Understanding these relationships could be crucial for developing interventions aimed at reducing obesity-related complications, including OSA.

The take home message from the study would be that since OSA is lifestyle related disorder, its importance to the sleep fraternity is gathering worldwide attention. Further, fragmented sleep which is a major characteristic of OSA leads to lower immunity thereby increasing the likelihood of infections. Therefore, considering the patient discomfort caused by the conventional diagnostic method (PSG), new non-invasive screening methods such as biomarker-based methods must be developed to ensure prompt detection. Towards this goal, profiling of miRNAs in a larger cohort followed by identification of their potential targets in clinical settings of OSA is warranted in order to bridge the gap in understanding the pathophysiology of OSA. Additional research is needed to explore how these regulatory signatures might contribute to the development of clinically relevant strategies for managing OSA. For instance, investigations of miRNAs and their targets among various populations like smokers vs. non-smokers, NAFLD, COPD, CVDs, etc. to discriminate OSA from other co-morbidities can be undertaken. Moreover, investigation of underlying mechanisms such as transcriptional regulation and mutational analysis of these miRNAs and their potential targets will be intriguing to open a new avenue for OSA theranostics for a better understanding of the relationship between OSA and metabolic complications. However, a major limitation of such study is small sample size and in-vitro cell culture model on OSA. The fact that OSA

like other diseases do not show any symptoms of severity in patients, it is mostly neglected so majority of population remains undiagnosed and unaware about the consequences of OSA. Also, polysomnography (PSG) being the only so far known diagnostic method, which involves lots of complications, expensive, overnight hospitalization etc., development of rapid detection techniques like DNA biosensors for OSA can be explored for detection of a pool of miRNA markers within clinical settings, which can be validated against AHI values from conventional PSG and RT-PCR data. Subsequently, by applying ML algorithms analyze the concentration of these target markers, coupled with pathological data, may collectively predict OSA and its severity and susceptibility to cormorbidities.

5. Conclusion

MiRNAs have emerged as potential biomarkers for OSA diagnosis, severity assessment and treatment response prediction. Additionally, targeting specific miRNAs holds promise as a therapeutic strategy for OSA management. Modulation of miRNA expression levels through pharmacological or genetic approaches may help to alleviate OSA-related complications by restoring normal cellular functions and signalling pathways. Our study focusses on the molecular mechanisms behind the dysregulation of miRNAs in OSA and their potential interactions at the gene level for targeting key *genes* or signaling pathways implicated in OSA pathophysiology through MD simulations study. More than 100 million Indians suffer from obesity and obesity-related metabolic and cardiovascular disorders. MiRNAs study holds promising roles as biomarkers, however, their role in OSA has not been investigated yet. The exploration of molecular signature such as miR-NAs, genes and proteins expressed in clinical conditions to generate a pathophysiological pattern in OSA is intriguing for therapeutic relevance.

CRediT authorship contribution statement

Gaganjyot Kaur Bakshi: Writing – original draft, Methodology. **Sartaj Khurana:** Writing – original draft, Methodology, Investigation, Data curation. **Shambhavee Srivastav:** Visualization. **Rohit Kumar:** Writing – review & editing, Resources. **Mukesh Chourasia:** Writing – review & editing, Resources, Methodology. **Sudeep Bose:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Institutional review board statement

This study was conducted in accordance with the Declaration of institutional ethics committee at Safdarjung Hospital, Delhi as well as Amity University, Noida.

Informed consent statement

Informed consent was obtained from all subjects involved in this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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