Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: January 31, 2024

Citation: Hall, SR., et al., 2024. Obstructive sleep apnea alters microRNA levels: Effects of continuous positive airway pressure. Medical Research Archives, [online] 12(1). https://doi.org/10.18103/mra.v12i1.4975

DOI: https://doi.org/10.18103/mra.v12i1.4975

ISSN: 2375-1924

RESEARCH ARTICLE

Obstructive sleep apnea alters microRNA levels: Effects of continuous positive airway pressure

SarahRose Hall[1], Stephanie Samani[1], Amelia Churillo[1], Lisa Freeburg[1], Oren Cohen[2], Kavya Devarakonda[2], Samira Khan[2], Kurt G. Barringhaus[3], Neomi Shah*[2], Francis G. Spinale*[1,4]

[1]Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, Columbia, SC

[2]Division of Pulmonary, Sleep, and Critical Care Medicine, Icahn School of Medicine at Mount Sinai, New York, NY

[3]Department of Cardiology, Prisma Health, Columbia, SC

[4]Columbia VA Health Care System, Columbia, SC

*Shared senior authorship

*cvctrc@uscmed.sc.edu

ABSTRACT

Background: Obstructive sleep apnea (OSA) has been linked to cytokine-mediated chronic inflammatory states. Continuous positive airway pressure (CPAP) is an established therapy for OSA, but its effects on inflammation remain unclear. A recent study from our group identified soluble cytokine receptors altered in OSA patients and modified by CPAP adherence. However, the upstream regulatory pathways responsible for these shifts in proinflammatory cascades with OSA and CPAP therapy remained unknown. Accordingly, this study mapped OSA and CPAP-modulated soluble cytokine receptors to specific microRNAs and then tested the hypothesis that OSA and CPAP adherence shift cytokine-related microRNA expression profiles.

Study Design: Plasma samples were collected from patients with OSA (n=50) at baseline and approximately 90 days after CPAP initiation and compared to referent control subjects (n=10). Patients with OSA were further divided into cohorts defined by adherence vs nonadherence to CPAP therapy. The microRNAs that mapped to soluble cytokine receptors of interest were subjected to quantitative polymerase chain reaction.

Results: At baseline, increased hsa-miR-15a-5p, hsa-miR-15b-5p, hsa-miR-16-5p, hsa-miR-195-5p, hsa-miR-424-5p, hsa-miR-223-3p, and hsa-miR-223-5p were observed in patients with OSA compared to controls ($p<0.05$). In CPAP adherent patients (n=22), hsa-miR233-3p and hsa-miR233-5p decreased at follow-up ($p<0.05$) whereas there was no change in miR levels from baseline in non-adherent CPAP patients (n=28). The miRs hsa-miR233-3p and hsa-miR233-5p mapped to both proinflammatory and innate immunity activation; the inflammasome.

Conclusion: A specific set of microRNAs, including hsa-miR233-3p and hsa-miR233-5p, may serve as a marker of inflammatory responses in patients with OSA, and be used to assess attenuation of inflammasome activation by CPAP.

Keywords: miR, microRNA, Soluble Cytokine Receptors, Inflammation, Continuous Positive Airway Pressure, Adherence, Obstructive Sleep Apnea, Cardiovascular Disease, Inflammasome

Introduction

Obstructive sleep apnea (OSA) is a prevalent medical issue affecting an estimated 17% of adults, and is considered a risk factor for many other pathologies, especially of cardiovascular nature.[1,2] OSA is characterized by repeated episodes of upper airway collapse and brief cessation of airflow.[3] This upper airway collapse has been associated with local pharyngeal inflammation.[4] Further, these episodes lead to sympathetic activation, perturbations in oxygen levels, accumulation of oxidative stress, and systemic inflammatory cytokine cascade activation.[5–12] Finally, cytokine activation has been proposed to enhance this sympathetic response, therefore causing a feed-forward mechanism for exacerbation of OSA.[2,13] A similar phenomenon has been documented in different inflammatory pathologies, such as coronary artery disease, hypertension, and other cardiovascular diseases.[2,13–17] More importantly, the cytokine activation with OSA has been linked to increased risk and or exacerbation of cardiovascular disease.[13-17] Thus, identifying the molecular pathways and checkpoints regarding inflammation with OSA, in particular cytokine activation, could potentially lead to new diagnostics and therapeutic strategies.

The current standard of care for OSA includes continuous positive air pressure (CPAP) therapy. CPAP therapy effectively maintains airflow throughout a patient's sleep by preventing the upper airway collapse that otherwise causes apnea-induced hypoxia. Past studies have identified that the intermittent hypoxia with OSA can cause local and systemic inflammation, and that CPAP may alter this process through attenuation of specific cytokine signaling cascades.[4-12, 15-23] However, the molecular basis for these changes in cytokine activation with OSA and CPAP remains poorly understood. Moreover, whether a molecular signature emerges with respect to regulation of inflammation which would hold diagnostic value in this context is also unexplored. The guiding hypothesis of the present study was that post-transcriptional pathways which regulate indices of inflammation can measured by plasma profiling in OSA patients receiving CPAP therapy.

MicroRNAs (miRs) are critical post-transcriptional regulators of cytokine production.[24-26] miRs can bind to cytokine transcripts and prevent translation, but can also regulate cytokine signaling indirectly by binding to transcripts of other molecules with inhibitory/anti-inflammatory properties. Moreover, cytokine activation can also alter miR profiles and thus form a potential feedback loop.[19,25] Although miR measurements in plasma have been performed in OSA patients previously,[16,17,19,27] these studies have not examined the effect of CPAP adherence using a focused miR array which may regulate cytokine production. Accordingly, the present study had two objectives. First, to develop a cytokine focused miR array using target prediction modeling,[28] which could be deployed in plasma samples from OSA patients. Through this approach, it was anticipated that a specific cassette of miRs would be altered in OSA patients when compared to referent control values. Second, quantify these miRs at baseline and following a 90 day follow-up period as a function of CPAP compliance. The results from this portion of the study would determine if miR levels which are altered with OSA are modifiable by CPAP.

Study Design and Methods

PATIENTS

Patients with treatment naïve OSA were recruited from pulmonary and sleep medicine clinics at the Mount Sinai Health System (New York, NY). OSA was confirmed by home sleep apnea testing using WatchPAT-200 and -300 devices (Itamar Medical, Caesarea, Israel). OSA was defined as a peripheral arterial tonometry–respiratory desaturation index (pRDI) greater than or equal to five respiratory events per hour of sleep. Pre-CPAP pRDI values were evaluated and categorized as mild, moderate, or severe based on the American Academy of Sleep Medicine definition.[29] Events were associated with either a 3% oxygen desaturation or an arousal (as measured by WatchPAT actigraphy). All participants gave written informed consent (Mount Sinai Institutional Research Board approval #18-00543). Following baseline blood sample collection, patients were prescribed clinically indicated CPAP treatment using auto-titrating devices with a pressure range set between 4 and 20 cm of H_2O. Adherence to CPAP was monitored remotely over a 90-day period. At 90 days (range: 80-100 days) a second blood sample was collected and final CPAP usage data was analyzed. For the purposes of providing a reference for miR profiling, non-OSA referent control samples (n=10) with an average age of 55$\pm$3 years (6 F/4M) were utilized as described in a previous study.[30] All referent control patients were prescreened and selected to exclude any chronic inflammatory/cardiovascular comorbidities.

miR EXTRACTION & PROFILING

The blood samples were placed on ice, centrifuged, plasma collected and aliquoted (500 uL) and maintained at -70degC until extraction was performed. Plasma was first thawed on ice and then subjected to miR extraction (miRNeasy Serum/Plasma Advanced Kit cat # 217204, Qiagen, Valencia, CA). The extracted miR pool was then subjected to quality analysis (Agilent RNA 6000 Nano Kit Santa Clara, CA). The miR pool was then reverse transcribed (miRCURY LNA RT Kit cat # 339340, Qiagen, Valencia, CA). This study utilized a miR array (YCA41416 Qiagen, Valencia, CA), which contained 17 individual miRs (**Table 1**) which targeted cytokine receptors that have previously been linked to OSA: soluble tumor necrosis factor receptor superfamily member 8 (sCD30), soluble epidermal growth factor receptor (sEGFR), soluble interleukin 1 receptor type I (sIL1RI), soluble interleukin 2 receptor alpha (sIL2Ra), soluble vascular endothelial growth factor 1 receptor (sVEGFR1), and soluble vascular endothelial growth factor 2 receptor (sVEGFR2).[5-12,15,16,20-23,31,32] These soluble cytokine receptors were mapped to miRs using Target Scan (Target Scan Human 7.2, Whitehead Institute for Biomedical Research).[28] In addition, miRs which mapped to innate immune activation, the nod-like receptor family pyrin domain containing 3 (NLRP3)-inflammasome was included.[33,34] The activation of the NLRP3 inflammasome has been reported in OSA patients.[32] The resulting cDNA from the miR primers was used for SYBR Green PCR (miScript SYBR Green Kit 4000 cat # 339347, Qiagen, Valencia, CA). Quantitative RT-PCR was performed (Bio-Rad CFX96 Touch) according to the vendor protocol. The maximum threshold cycle (C_T) for detection was set at 35 C_Ts. miRs that cycled above 35 C_Ts were excluded from the remainder of PCR analysis. C_T values of the mean for Qiagen interplate calibrator was used to adjust for plate-to-plate variation.

DATA ANALYSIS

CPAP adherence was as CPAP usage of greater than or equal to 4 hours per night on 70% of nights during the study period.[29,35] The demographics of CPAP adherent and non-adherent cohorts were examined using a frequency distribution and chi-squared analysis. Age comparisons were performed using a two-sample t-test.

Relative miR levels were first computed as delta CT (dCT) values using an internal reference miR, hsa-miR451a. The delta dCT (ddCT) was calculated as the difference in analyte dCT value from that of the mean referent control value. The fold change was then calculated using the 2^{-ddCT} approach as described previously,[36,37] for baseline and for the 90-day follow-up time point. A paired t-test was performed to detect changes between samples taken at baseline versus after 90-days of CPAP therapy, with the adherent and non-adherent groups analyzed separately. An independent samples t-test was performed to compare 90-day values in terms of CPAP compliance. Finally, a multivariate analysis of variance (ANOVA) was performed with miR expression levels as the output variable of interest, considering adherence and time as treatment effects. A p value of < 0.05 was used for statistical significance. IBM SPSS Statistics 28 (IBM, New York USA) software was used for all analysis. Summary data are presented as the mean $\pm$ standard error of the mean.

Results

Patient demographics for the study are summarized in **Table 2**. Age (51±2, 46±2 yo), body mass index (32±1, 32±1), and sex distribution (27%, 29% female) were similar between CPAP adherent and non-adherent groups, respectively ($p>0.05$). Mean peripheral arterial tonometry–respiratory desaturation index (pRDI) for the total sample set was 30.4. There was no significant difference detected in baseline pRDI between patients adherent and non-adherent to CPAP therapy (29±3 vs 32±4 respectively, $p=0.377$).

Using quantitative PCR and the miRs identified in **Table 1**, 10 of the 17 miRs did not meet the cycle threshold in the referent control samples and thus could not be properly indexed. Of the 7 miRs which did cycle, these were markedly changed in the OSA patients at baseline (**Table 3**). Notably, a robust increase in hsa-miR15b-5p, hsa-miR223-3p, and hsa-miR223-5p were observed. At a 90 day follow-up interval, these 7 miRs remained elevated in the OSA patients as a group. However, hsa-miR223-3p, and hsa-miR223-5p were decreased from baseline values in those OSA patients compliant to CPAP. In contrast, these miRs remained unchanged from baseline values in the OSA patients not compliant with CPAP. Using these 7 miRs, multivariate ANOVA did not reveal any treatment effect of time nor adherence.

Table 1. A series of 17 miRs were selected for study as these mapped to specific inflammatory pathways which have been identified previously in OSA patients.

Table 1. MicroRNAs with High Probability of Targeting Cytokine Cascade																	
	hsa-miR15a-5p	hsa-miR15b-5p	hsa-miR16-5p	hsa-miR195-5p	hsa-miR378g	hsa-miR424-5p	hsa-miR497-5p	hsa-miR129-5p	hsa-miR141-3p	hsa-miR186-3p	hsa-miR190a-3p	hsa-miR200a-3p	hsa-miR204-3p	hsa-miR211-3p	hsa-miR367-5p	hsa-miR223-3p	hsa-miR223-5p
sEGFR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
sIL1RI	x	x	x	x	x	x	x	x		x	x		x	x	x	x	x
sIL2Ra	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
sVEGFR1	x	x	x	x	x	x	x	x	x		x	x	x	x	x		
sVEGFR2	x	x	x	x	x	x	x	x	x	x	x	x			x		
sCD30					x				x	x		x	x	x			

Table 2. Patient demographics for the OSA patients in which miR plasma profiling was performed.

Table 2. Patient Demographics in OSA Patients	
Patients, n	50
Female, n (%)	14 (28%)
Male, n (%)	36 (72%)
Age + SEM	48±2
BMI + SEM	32±1
Race, n (%)	
White	24 (48%)
Black	11 (22%)
Asian	4 (8%)
Unsure	6 (12%)
Other	5 (10%)
Ethnicity, n (%)	
Hispanic	18 (36%)
Other	32 (64%)
pRDI value, n (%)	
Mild (0-15)	9 (18%)
Moderate (15-30)	19 (38%)
Severe (30+)	22 (44%)

pRDI = Respiratory Desaturation Index; CPAP = Continuous Positive Airway Pressure

Table 3. Seven miRs were identified to be increased with OSA compared to referent controls and 2 miRs changed as a function of CPAP compliance.

Table 3. Changes in miRs as a function of OSA and CPAP Compliance					
	RC	Baseline OSA total	90 days OSA total	90 days Compliant to CPAP	90 days Non-compliant to CPAP
hsa-miR-15a-5p	1.04 ± 0.09	16.61 ± 1.67*	14.87 ± 1.71*	14.46 ± 2.31*	15.20 ± 1.54*
hsa-miR-15b-5p	1.16 ± 0.20	79.76 ± 8.78*	67.29 ± 3.52*	69.30 ± 9.87*	77.79 ± 7.59*
hsa-miR-16-5p	1.08 ± 0.11	16.10 ± 1.76*	15.61 ± 1.58*	13.27 ± 1.74*	16.58 ± 1.68*
hsa-miR-195-5p	1.26 ± 0.42	23.07 ± 4.08*	17.07 ± 2.73*	17.18 ± 1.47*	22.62 ± 3.88*
hsa-miR-424-5p	1.14 ± 0.19	17.11 ± 2.26*	14.91 ± 2.64*	17.42 ± 3.12*	20.73 ± 3.30*
hsa-miR-223-3p	1.27 ± 0.27	173.15 ± 16.19*	203.26 ± 20.93*†	172.97 ± 59.07*†	225.24 ± 104.44*
hsa-miR-223-5p	1.01 ± 0.07	169.09 ± 13.88*	146.11 ± 6.03*†	133.01 ± 14.37*†	151.23 ± 19.68*
Total Sample Size (n)	10	50	50	22	28

OSA = Obstructive Sleep Apnea, CPAP = Continuous Positive Airway Pressure, RC = Referent Control

* P < 0.05 by t-test vs referent control

† P < 0.05 by paired t-test vs baseline

Discussion

In the present study, we utilized an array of miRs which mapped to soluble cytokine receptors that have demonstrated involvement in chronic cardiopulmonary inflammation and/or the inflammasome. From this approach, 7 miRs were increased in OSA patients compared to referent control values. Following a study interval of 90 days with CPAP, those patients which were designated as compliant to CPAP, 2 of these miRs decreased from baseline values. The unique findings of the present study is that a specific cassette of miRs, which map to specific inflammatory cascades, were elevated in OSA patients and only 2 appeared to be influenced by CPAP treatment. These findings suggest that plasma miR profiling may provide insight into the underlying molecular mechanisms associated with inflammation in OSA patients and that a select number of miRs may hold value as a biomarker for assessing CPAP effectiveness/compliance.

Several studies have linked inflammation, in particular cytokine activation, to OSA, and identified shifts in relative cytokine levels in OSA patients as a function of CPAP use and adherence.[5-7,9-12,15,20,31,32] However, the upstream molecular checkpoints for these inflammatory pathways, particularly dynamic post-transcriptional regulation by miRs, remain relatively unexplored in this context. Past studies that have examined miR levels in the context of OSA have either focused on a specific miR or were not stratified with respect to CPAP use and adherence.[17,19] For example, Li and colleagues reported an increase in miR-664a-3p in patients with OSA and atherosclerosis, suggesting a common regulatory pathway may underlie both conditions.[17] MiR-664a-3p has been linked to the downregulation of cytokines such as interleukin 6, tumor necrosis factor, and mitogen-activated protein kinase 1.[38] However, the present study did not examine miR-664a-3p as it did not fit the criteria of mapping to a cytokine receptor pathway identified previously in OSA patients.[5–7,9-12,15,20,31,32] Consistent with the present study, a past report identified that mir-15b-5p was increased in OSA patients and linked to a canonical cytokine signaling pathway, NF-kB.[19] However, this past study did not examine changes in miR levels as a function of CPAP adherence. A past study by Santamaria-Martos et al. did examine multiple miRs in OSA and identified that miR hsa-miR-345 was reduced following six months of CPAP.[39] However, this previous study did not index miR levels to referent control levels and did not examine miR levels as a function of CPAP adherence. The present study was hypothesis driven with respect to selecting an array of miRs specific to inflammatory cytokine pathways which have been identified to be activated in OSA patients. As such, hsa-miR-345 was not included in the present analysis but may warrant inclusion in a future study.

Of the 7 specific miRs which were increased in OSA patients, the majority of these mapped with high probability to the soluble cytokine receptors sEGFR, sIL1R1, sIL2Ra, sVEGFR1, and sVEGFR2.[33] Prior studies by our group have linked these soluble cytokine receptors to the chronic inflammation in patients with cardiovascular disease.[30,40] Several of these soluble cytokines, such as the IL1 and VEGF receptors have been reported in OSA patients.[40-42] Past studies have identified an increased risk of cardiovascular disease in OSA patients which may be due to the activation of these proinflammatory cytokines.[1,10,12,13,21]

However, a direct causal relationship has not been clearly established. For example, a reduction in cytokine levels is not a uniform finding in OSA patients following a period of CPAP therapy.[6,9,10,12,20,31] In the present study, these specific miRs which potentially regulate these soluble cytokine receptors, appeared to be unaffected by a period of CPAP therapy. Thus, it could be postulated that the persistent elevation of these specific miRs is due to a continued underlying inflammatory process in OSA patients which was unaffected by CPAP. However, it must be recognized that the results from the present study are associative and a direct mechanistic relationship between the elevation in a specific miR to a specific cytokine pathway was not established. Nevertheless, some of the miRs identified to be increased in OSA patients been demonstrated to modulate various cytokine pathways.[43-45] For example, a prior study by Li et al. demonstrated hsa-miR-15a-5p can regulate the STAT3/CX3CL1/NF-kB pathway.[43] In another study, silencing hsa-miR-15b-5p may induce cellular apoptosis in in the context of a chronic inflammatory state.[45] When another miR identified to be increased in OSA patients, hsa-miR-195-5p, was silenced in a rat model profibrotic signaling pathways were attenuated.[46] Increased levels of hsa-miR-424-5p, which was identified in OSA patients, has been implicated in the regulation cell division, migration, and cell cycle regulation.[47] Moreover, hsa-miR-424-5p has been shown to inhibit the inflammatory activity of interleukins and NF-kB.[48] Finally, the miR hsa-miR-16-5p, is broadly thought of as having tumor suppressor activity in addition to negatively regulate various cytokines associated with chronic inflammatory states.[49]

Another unique finding of the present study was a relative reduction in hsa-miR-223-3p and hsa-miR-223-5 following 90 days of CPAP adherence. These miRs map with high probability to soluble receptors of the IL-1 and IL-2 cytokine families, which are involved in T-cell recruitment and inflammatory responses.[50-54] Increased activity levels of both cytokines have been associated with many autoimmune, vascular, and malignant pathologies; as such these cytokines have been targeted for numerous therapies. Hsa-miR-223-3p and hsa-miR-223-5 are also tightly linked to the NLRP3 inflammatory cascade.[55-57] Elevated NLRP3 and inflammasome activation have been identified as the link between the intermittent hypoxia observed in patients with OSA and chronic inflammatory pathology.[32] Our finding that hsa-miR-223-3p and hsa-miR-223-5 are reduced in patients with OSA who are adherent to CPAP therapy suggests that CPAP therapy reduces NLRP3 levels inflammasome activity. However, the present study did not quantify NLRP3/inflammasome pathway and thus this remains speculative. Nevertheless, these findings suggest that hsa-miR-223-3p and hsa-miR-223-5 are may be strong candidates to be included in a biomarker matrix in terms of assessing the effectiveness/compliance of CPAP in OSA patients.

LIMITATIONS

As with most studies that examine miR profiles in a clinical disease state, we used a peripheral blood sample, which may not directly reflect local miR changes within the pulmonary system. Additionally, the present study used a small set of referent normal subjects. However these values were simply used for indexing purposes. The present study did not examine the effect of comorbidities or covariates on

miR profiles as a larger sample size would have been required to perform subset analysis. However, the findings of the present study strongly support a future larger OSA patient study with serial measures and CPAP adherence, race/ethnicity, and sex as covariates.

SUMMARY

The present study identified a specific cassette of inflammation-related miRs that were increased in patients with OSA, and two miRs that fell as a function of CPAP adherence. These results indicate that in the context of OSA, CPAP use can modify post-transcriptional regulators of key inflammatory processes likely to contribute to the development and exacerbation of OSA.

Conflicts of Interest:

None

Funding:

This study was supported by NIH grant (R01HL130972; Spinale; R01HL143221; Shah), VA Merit Funding (5I01-BX000168, 5I01-BX005320; Spinale).

Acknowledgements

None

References:

1. Marin JM, Agusti A, Villar I, et al. Association between treated and untreated obstructive sleep apnea and risk of hypertension. *JAMA*. 2012;307(20):2169-2176. doi:10.1001/jama.2012.3418

2. Montesi SB, Bajwa EK, Malhotra A. Biomarkers of sleep apnea. *Chest*. 2012; 142(1):239-245. doi:10.1378/chest.11-2322

3. AASM scoring manual - American Academy of Sleep Medicine. American Academy of Sleep Medicine – Association for Sleep Clinicians and Researchers. June 7, 2023. https://aasm.org/clinical-resources/scoring-manual/.

4. Cohen O, Kaufman AE, Choi H, et al. Pharyngeal Inflammation on Positron Emission Tomography/Magnetic Resonance Imaging Before and After Obstructive Sleep Apnea Treatment. *Ann Am Thorac Soc*. 2023;20(4):574-583. doi:10.1513/AnnalsATS.202207-594OC

5. Kritikou I, Basta M, Vgontzas AN, et al. Sleep apnoea, sleepiness, inflammation and insulin resistance in middle-aged males and females. *Eur Respir J*. 2014;43(1):145-155. doi:10.1183/09031936.00126712

6. Kohler M, Ayers L, Pepperell JC, et al. Effects of continuous positive airway pressure on systemic inflammation in patients with moderate to severe obstructive sleep apnoea: a randomised controlled trial. *Thorax*. 2009; 64(1):67-73. doi:10.1136/thx.2008.097931

7. Wali SO, Al-Mughales J, Alhejaili F, et al. The utility of proinflammatory markers in patients with obstructive sleep apnea [published correction appears in Sleep Breath. 2022 Sep; 26(3):1505]. *Sleep Breath*. 2021;25(2):545-553. doi:10.1007/s11325-020-02149-3

8. Vgontzas AN, Zoumakis E, Bixler EO, et al. Selective effects of CPAP on sleep apnoea-associated manifestations. *Eur J Clin Invest*. 2008;38(8):585-595. doi:10.1111/j.1365-2362.2008.01984.x

9. Karamanlı H, Özol D, Ugur KS, et al. Influence of CPAP treatment on airway and systemic inflammation in OSAS patients. *Sleep Breath*. 2014;18(2):251-256. doi:10.1007/s11325-012-0761-8

10. Thunström E, Glantz H, Yucel-Lindberg T, Lindberg K, Saygin M, Peker Y. CPAP Does Not Reduce Inflammatory Biomarkers in Patients With Coronary Artery Disease and Nonsleepy Obstructive Sleep Apnea: A Randomized Controlled Trial. *Sleep*. 2019; 42(2):10.1093/sleep/zsy241. doi:10.1093/sleep/zsy241

11. Lin CC, Liaw SF, Chiu CH, Chen WJ, Lin MW, Chang FT. Effects of nasal CPAP on exhaled SIRT1 and tumor necrosis factor-α in patients with obstructive sleep apnea. *Respir Physiol Neurobiol*. 2016;228:39-46. doi:10.1016/j.resp.2016.03.001

12. Jin F, Liu J, Zhang X, et al. Effect of continuous positive airway pressure therapy on inflammatory cytokines and atherosclerosis in patients with obstructive sleep apnea syndrome. *Mol Med Rep*. 2017;16(5):6334-6339. doi:10.3892/mmr.2017.7399

13. Basner RC. Cardiovascular morbidity and obstructive sleep apnea [published correction appears in N Engl J Med. 2014 Jul 10;371(2):189]. *N Engl J Med*. 2014;370(24):2339-2341. doi:10.1056/NEJMe1404501

14. Punjabi NM. The epidemiology of adult obstructive sleep apnea. *Proc Am Thorac Soc*. 2008;5(2):136-143. doi:10.1513/pats.200709-155MG

15. Unnikrishnan D, Jun J, Polotsky V. Inflammation in sleep apnea: an update. *Rev Endocr Metab Disord*. 2015;16(1):25-34. doi:10.1007/s11154-014-9304-x

16. Chen YC, Hsu PY, Su MC, et al. miR-21-5p Under-Expression in Patients with Obstructive Sleep Apnea Modulates Intermittent Hypoxia with Re-Oxygenation-Induced-Cell Apoptosis and Cytotoxicity by Targeting Pro-Inflammatory TNF-α-TLR4 Signaling. *Int J Mol Sci*. 2020;21(3):999. Published 2020 Feb 3. doi:10.3390/ijms21030999

17. Li K, Chen Z, Qin Y, Wei Y. MiR-664a-3p expression in patients with obstructive sleep apnea: A potential marker of atherosclerosis. *Medicine (Baltimore)*. 2018;97(6):e9813. doi:10.1097/MD.0000000000009813

18. Bonsignore MR, McNicholas WT, Montserrat JM, Eckel J. Adipose tissue in obesity and obstructive sleep apnoea. *Eur Respir J*. 2012;39(3):746-767. doi:10.1183/09031936.00047010

19. Chen YC, Hsu PY, Su MC, et al. MicroRNA Sequencing Analysis in Obstructive Sleep Apnea and Depression: Anti-Oxidant and MAOA-Inhibiting Effects of miR-15b-5p and miR-92b-3p through Targeting PTGS1-NF-κB-SP1 Signaling. *Antioxidants (Basel)*. 2021;10(11):1854. Published 2021 Nov 22. doi:10.3390/antiox10111854

20. Steiropoulos P, Kotsianidis I, Nena E, et al. Long-term effect of continuous positive airway pressure therapy on inflammation markers of patients with obstructive sleep apnea syndrome. *Sleep*. 2009;32(4):537-543. doi:10.1093/sleep/32.4.537

21. Colish J, Walker JR, Elmayergi N, et al. Obstructive sleep apnea: effects of continuous positive airway pressure on cardiac remodeling as assessed by cardiac biomarkers, echocardiography, and cardiac MRI. *Chest*. 2012;141(3):674-681. doi:10.1378/chest.11-0615

22. Cifçi N, Uyar M, Elbek O, Süyür H, Ekinci E. Impact of CPAP treatment on cardiac biomarkers and pro-BNP in obstructive sleep apnea syndrome. *Sleep Breath*. 2010;14(3):241-244. doi:10.1007/s11325-009-0306-y

23. Fleming WE, Holty JC, Bogan RK, et al. Use of blood biomarkers to screen for obstructive sleep apnea. *Nat Sci Sleep*. 2018;10:159-167. Published 2018 Jun 14. doi:10.2147/NSS.S164488

24. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics*. 2010;11(7):537-561. doi:10.2174/138920210793175895

25. Garavelli S, De Rosa V, de Candia P. The Multifaceted Interface Between Cytokines and microRNAs: An Ancient Mechanism to Regulate the Good and the Bad of Inflammation. *Front Immunol*. 2018;9:3012. Published 2018 Dec 21. doi:10.3389/fimmu.2018.03012

26. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15-20. doi:10.1016/j.cell.2004.12.035

27. Goody PR, Nachtsheim L, Hosen MR, et al. Analysis of nocturnal, hypoxia-induced miRNAs in sleep apnea patients. *PLoS One*. 2022;17(3):e0263747. Published 2022 Mar 4. doi:10.1371/journal.pone.0263747

28. TargetScanHuman 8.0. Accessed January 3, 2023. https://www.targetscan.org/vert_80/

29. Kapur VK, Auckley DH, Chowdhuri S, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea:

An American Academy of Sleep Medicine Clinical Practice Guideline. *J Clin Sleep Med*. 2017;13(3):479-504. Published 2017 Mar 15. doi:10.5664/jcsm.6506

30. Spinale FG, Meyer TE, Stolen CM, et al. Development of a biomarker panel to predict cardiac resynchronization therapy response: Results from the SMART-AV trial. *Heart Rhythm*. 2019;16(5):743-753. doi:10.1016/j.hrthm.2018.11.026

31. Unüvar Doğan F, Yosunkaya S, Kuzu Okur H, Can U. Relationships between Obstructive Sleep Apnea Syndrome, Continuous Positive Airway Pressure Treatment, and Inflammatory Cytokines. *Sleep Disord*. 2014;2014:518920. doi:10.1155/2014/518920

32. Díaz-García E, García-Tovar S, Alfaro E, et al. Inflammasome Activation: A Keystone of Proinflammatory Response in Obstructive Sleep Apnea. *Am J Respir Crit Care Med*. 2022;205(11):1337-1348. doi:10.1164/rccm.202106-1445OC

33. Blevins HM, Xu Y, Biby S, Zhang S. The NLRP3 Inflammasome Pathway: A Review of Mechanisms and Inhibitors for the Treatment of Inflammatory Diseases. *Front Aging Neurosci*. 2022;14:879021. Published 2022 Jun 10. doi:10.3389/fnagi.2022.879021

34. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int J Mol Sci*. 2019;20(13):3328. Published 2019 Jul 6. doi:10.3390/ijms20133328

35. Sánchez-de-la-Torre M, Sánchez-de-la-Torre A, Bertran S, et al. Effect of obstructive sleep apnoea and its treatment with continuous positive airway pressure on the prevalence of cardiovascular events in patients with acute coronary syndrome (ISAACC study): a randomised controlled trial. *Lancet Respir Med*. 2020;8(4):359-367. doi:10.1016/S2213-2600(19)30271-1

36. Oatmen KE, Toro-Salazar OH, Hauser K, Zellars KN, Mason KC, Hor K, Gillan E, Zeiss CJ, Gatti DM, Spinale FG. Identification of a novel microRNA profile in pediatric patients with cancer treated with anthracycline chemotherapy. Am J Physiol Heart Circ Physiol. 2018 Nov 1;315(5):H1443-H1452. doi: 10.1152/ajpheart.00252.2018. Epub 2018 Aug 24. PMID: 30141982.

37. Zile MR, Mehurg SM, Arroyo JE, Stroud RE, DeSantis SM, Spinale FG. Relationship between the temporal profile of plasma microRNA and left ventricular remodeling in patients after myocardial infarction. Circ Cardiovasc Genet. 2011 Dec;4(6):614-9. doi: 10.1161/CIRCGENETICS.111.959841. Epub 2011 Sep 28. PMID: 21956146; PMCID: PMC3535326.

38. Zhang Q, Xiao X, Li M, et al. Acarbose reduces blood glucose by activating miR-10a-5p and miR-664 in diabetic rats. *PLoS One*. 2013;8(11):e79697. Published 2013 Nov 18. doi:10.1371/journal.pone.0079697

39. Santamaria-Martos F, Benítez I, Ortega F, et al. Circulating microRNA profile as a potential biomarker for obstructive sleep apnea diagnosis. *Sci Rep*. 2019;9(1):13456. Published 2019 Sep 17. doi:10.1038/s41598-019-49940-1

40. Young BD, Moreland H, Oatmen KE, et al. Cytokine Signaling and Matrix Remodeling Pathways Associated with Cardiac Sarcoidosis Disease Activity Defined Using FDG PET Imaging. doi:10.1536/ihj.21-164

41. Fiedorczuk P, Polecka A, Walasek M, Olszewska E. Potential Diagnostic and

Monitoring Biomarkers of Obstructive Sleep Apnea-Umbrella Review of Meta-Analyses. J Clin Med. 2022 Dec 21;12(1):60. doi: 10.3390/jcm12010060. PMID: 36614858; PMCID: PMC9821668.

42. Yi M, Zhao W, Fei Q, Tan Y, Liu K, Chen Z, Zhang Y. Causal analysis between altered levels of interleukins and obstructive sleep apnea. Front Immunol. 2022 Jul 27;13:888644. doi: 10.3389/fimmu.2022.888644. PMID: 35967324; PMCID: PMC9363575.

43. Li H, Zhang HM, Fan LJ, et al. STAT3/miR-15a-5p/CX3CL1 Loop Regulates Proliferation and Migration of Vascular Endothelial Cells in Atherosclerosis. *Int J Med Sci*. 2021;18(4):964-974. Published 2021 Jan 1. doi:10.7150/ijms.49460

44.Chava S, Reynolds CP, Pathania AS, et al. miR-15a-5p, miR-15b-5p, and miR-16-5p inhibit tumor progression by directly targeting MYCN in neuroblastoma. *Mol Oncol*. 2020;14(1):180-196. doi:10.1002/1878-0261.12588

45. Ghafouri-Fard S, Khoshbakht T, Hussen BM, Jamal HH, Taheri M, Hajiesmaeili M. A Comprehensive Review on Function of miR-15b-5p in Malignant and Non-Malignant Disorders. *Front Oncol*. 2022;12:870996. Published 2022 May 2. doi:10.3389/fonc.2022.870996

46. Ding H, Yao J, Xie H, et al. MicroRNA-195-5p Downregulation Inhibits Endothelial Mesenchymal Transition and Myocardial Fibrosis in Diabetic Cardiomyopathy by Targeting Smad7 and Inhibiting Transforming Growth Factor Beta 1-Smads-Snail Pathway. *Front Physiol*. 2021;12:709123. Published 2021 Sep 30. doi:10.3389/fphys.2021.709123

47. Li Y, Liu J, Hu W, et al. miR-424-5p Promotes Proliferation, Migration and Invasion of Laryngeal Squamous Cell Carcinoma. *Onco Targets Ther*. 2019;12:10441-10453. Published 2019 Nov 29. doi:10.2147/OTT.S224325

48. Li Z, Wang T, Yu Y. miR-424 inhibits apoptosis and inflammatory responses induced by sevoflurane through TLR4/MyD88/NF-κB pathway. *BMC Anesthesiol*. 2022;22(1):52. Published 2022 Feb 23. doi:10.1186/s12871-022-01590-z

49. Ghafouri-Fard S, Khoshbakht T, Hussen BM, Abdullah ST, Taheri M, Samadian M. A review on the role of mir-16-5p in the carcinogenesis. *Cancer Cell Int*. 2022;22(1):1-11. doi:10.1186/S12935-022-02754-0/TABLES/3

50. Szekely Y, Arbel Y. A Review of Interleukin-1 in Heart Disease: Where Do We Stand Today? *Cardiol Ther*. 2018;7(1):25-44. doi:10.1007/s40119-018-0104-3

51. Cavalli G, Colafrancesco S, Emmi G, et al. Interleukin 1α: a comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases. *Autoimmun Rev*. 2021;20(3):102763. doi:10.1016/j.autrev.2021.102763

52. Damoiseaux J. The IL-2 - IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor. *Clin Immunol*. 2020; 218:108515. doi:10.1016/j.clim.2020.108515

53. Li B, Guo Q, Wang Y, et al. Increased Serum Interleukin-2 Levels Are Associated with Abnormal Peripheral Blood Natural Killer Cell Levels in Patients with Active Rheumatoid Arthritis. *Mediators Inflamm*. 2020; 2020:6108342. Published 2020 Sep 15. doi:10.1155/2020/6108342

54. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood*. 2011;117(14):3720-3732. doi:10.1182/blood-2010-07-273417

55. Zhang M, Wang L, Huang S, He X. MicroRNA-223 targets NLRP3 to relieve inflammation and alleviate spinal cord injury. Life Sci. 2020 Aug 1;254:117796. doi: 10.1016/j.lfs.2020.117796. Epub 2020 May 14. PMID: 32417375.

56. Qin D, Wang X, Li Y, Yang L, Wang R, Peng J, Essandoh K, Mu X, Peng T, Han Q, Yu KJ, Fan GC. MicroRNA-223-5p and -3p Cooperatively Suppress Necroptosis in Ischemic/Reperfused Hearts. J Biol Chem. 2016 Sep 16;291(38):20247-59. doi: 10.1074/jbc.M116.732735. Epub 2016 Aug 8. PMID: 27502281; PMCID: PMC5025706.

57. Long FQ, Kou CX, Li K, Wu J, Wang QQ. MiR-223-3p inhibits rTp17-induced inflammasome activation and pyroptosis by targeting NLRP3. J Cell Mol Med. 2020 Dec;24(24):14405-14414. doi: 10.1111/jcmm.16061. Epub 2020 Nov 3. PMID: 33145937; PMCID: PMC7754033.