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REVIEW ARTICLES

Epigenetics of obstructive sleep apnea syndrome: a systematic review

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Study Objectives: Obstructive sleep apnea (OSA) is a chronic and widely prevalent disease associated with multiple health disorders. Current diagnostic strategies for OSA are limited because of cost, time, and access. Epigenetic signatures offer insight into the relationships between disease and environment and could play a significant role in developing both diagnostic and therapeutic tools for OSA. In the current study, a systematic literature search was conducted to investigate the existing evidence of OSA-associated epigenetic modifications.

Methods: A systematic literature search was performed using electronic academic databases including PubMed, CINAHL, Scopus, Embase, EBM Reviews, and Web of Science. However, the current study focused on screening for original, English-language articles pertaining to OSA and associated epigenetic mechanisms. To produce unbiased results, screening was performed independently by authors.

Results: We identified 2,944 publications in our systematic search. Among them, 65 research articles were related to OS A-associated differential gene expression, genetic variation, and epigenetic modifications. Although these 65 articles were considered for full manuscript review, only 12 articles met the criteria of OSA-associated epigenetic modifications in human and animal models. Human patients with OSA had unique epigenetic changes compared to healthy control patients and, interestingly, epigenetic signatures were commonly identified in genes associated with metabolic and inflammatory pathways.

Conclusions: Although the available studies are limited, this research provides novel insights for the development of epigenetic markers for the diagnosis and treatment of OSA. Thorough genome-wide investigations will be required to develop cost-effective, robust biomarkers for the identification of OSA among children and adults. Here, we offer a study design for such efforts.

Keywords: obstructive sleep apnea, biomarkers, epigenetics, systematic review

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INTRODUCTION

Next-generation diagnostics and therapeutics could provide safe and effective health care that improves patient outcomes and reduces the cost of medical care. Although there are some political, economic, and educational challenges in the development and implementation of alternative diagnostics and treatments in health care, it has been shown that newer methods, such as gene therapy, hold promise for better outcomes of highly prevalent disorders such as cancer, heart disease, and diabetes. Furthermore, the results of genetic tests including gene expression changes, genetic variation, and epigenetic signatures potentially serve as biomarkers for disease. Although sleep disorders are highly prevalent in modern society, alternative diagnostics are not readily available. Health care providers still predominantly rely on polysomnograms for diagnosis. Because of the limited availability of sleep medicine providers, there is an increased demand for alternative diagnostic instruments for sleep disorders, especially for children and vulnerable populations.

Obstructive sleep apnea (OSA) has been reported in 2%–5% of children[1] and in 35% of adults between ages 30 and 69 years.[2] The financial weight of OSA is significant, especially because of the lifetime health burden in undiagnosed patients. In 2015, the estimated cost to the U.S. health care system for adults with undiagnosed OSA was 12 times greater ($149.6 billion) than the cost for diagnosis and treatment of OSA ($12.4 billion).[3] Untreated OSA is associated with increased major adverse cardiac events, coronary heart disease, stroke, cardiac death, and all-cause mortality.[4] The reasons are poorly understood. However, the existing literature suggests that these risks for patients with OSA could be caused by the dysregulation of multiple biological pathways, including those that lead to the dysregulation of sympathetic activation,[5] vascular endothelial dysfunction,[6,7] systemic inflammation,[8] and oxidative stress.[9] Although polysomnograms are the gold standard for the diagnosis of OSA, this method does not allow clinicians to stratify chronic risks from OSA or to delineate the systems that could be primarily affected, depending on the variability in underlying pathologies. With these elements in mind, genetic and genomic markers may serve as an alternative and

more descriptive diagnostic tool to identify OSA and the associated health risks.

Recent omics studies suggest that patients with OSA show a differential expression of genes in the oxidative stress response and inflammatory pathways.[10,11] Coordinated, parallel observations of disease-specific DNA methylation in the genomes of pediatric and adult patients with OSA suggest that epigenetic modifications could play a significant role in OSA diagnosis and treatment.[6,12,13] Epigenetic modifications are functionally relevant changes to the genome that impact the regulation of gene expression without alteration of the original DNA sequence. These epigenetic signatures have served as potential biomarkers for cancer[14,15] and neurodegenerative disorders,[16] among other diseases. We hypothesize that epigenetic signatures could serve as cost-effective diagnostic instruments for the diagnosis of OSA-associated health conditions. In this study, we conducted a systematic review of the literature to investigate whether epigenetic signatures could serve as potential biomarkers for OSA.

METHODS

Literature selection

Our systematic literature review was conducted using established Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. A combination of keywords was used to retrieve the available literature on OSA and associated genetic studies using 6 of the most commonly used academic databases/search engines: PubMed, CINAHL, Scopus, Embase, EBM Reviews, and Web of Science. The search was conducted for manuscripts published through September 2020. This search included 2 primary components:

(1) obstructive sleep apnea (OR) OSA (OR) sleep-disordered breathing (OR) OSAHS (OR) sleep apnea hypopnea syndrome (OR) obesity hypoventilation syndrome (OR) sleep apnea, obstructive (AND)
(2) RNA (OR) ribonucleic acid (OR) RNA expression (OR) RNA expression signature (OR) genetic transcription (OR) transcriptome (OR) epigenetic (OR) epigenomics (OR) epigenetic modification (OR) epigenetic alteration (OR) transcription, genetic

Study inclusion and exclusion criteria

A systematic literature search was conducted independently by 2 authors (BAL and DFS) to produce unbiased results. After removing duplicates, we identified 2,944 articles from the 6 databases used in the current study. Included or excluded studies were initially screened based on the titles and abstracts. For the current study, we focused on English-language studies involving human and animal models. Studies not in English or pertaining to unrelated diseases were excluded. After this preliminary screening, all authors reviewed the findings together and came to a consensus on which articles should be included or excluded. Based on the initial screening, another thorough screening was performed by authors independently by reading individual article abstracts. Review articles and individual abstract publications were excluded in the systematic literature search. Sixty-five articles were considered for full manuscript review, and 12 among them were prospective articles associated with OSA and epigenetics.

Data extraction

The data were extracted by independent investigators after a thorough screening and discussion by all coauthors. We identified 12 eligible articles in humans and rodents. The data from eligible articles were extracted and discussed in the manuscript review, including author names, year of publication, specimen information, study population, analysis technique, extent of epigenetic evaluation (specific vs whole genome), and major findings (**Table 1** and **Table 2**).

RESULTS

Systematic literature search

Our systematic literature search for OSA and associated genetic studies through PubMed, CINAHL, Scopus, Embase, EBM Review, and Web of Science identified 2,944 articles. After applying our inclusion and exclusion criteria, we selected 65 articles for full manuscript review, and 12 of them met our primary criteria. The flow diagram outlining this systematic review search process is outlined in **Figure 1**.

Epigenetic modifications occurring from OSA or animal models representing OSA were studied in 12 different articles (6 human, 6 rodents). Five of the human studies utilized peripheral blood samples,[6,12,17–19] and 1 used visceral fat samples[13] to understand the epigenetic signatures in OSA (**Table 1**). Similarly, in rodent studies (**Table 2**), 3 articles showed intermittent hypoxia-associated epigenetic modifications in peripheral blood samples:[20–22] 1 in visceral fat,[23] 1 in adipocytes of perivascular adipose tissue,[24] and 1 in aorta-specific macrophages.[25] Among the 12 articles, 9 articles reported genome-wide epigenetic signatures, whereas 3 articles focused on DNA surrounding individual candidate genes. An overview of these studies with key observations, including target genes and other associated clinical conditions, is reported in **Table 1** and **Table 2**.

Metabolism

Metabolism is one of the most common biological pathways affected by epigenetic modification as a result of chronic intermittent hypoxia/OSA. In patients with OSA, Chen, Yang, and colleagues[13] identified differential promoter methylation in genes associated with metabolism including actin alpha 1, histone deacetylase 2, and small ubiquitin-related modifier 1. Similarly, in rodent models, late gestational intermittent hypoxia was associated with differential promoter methylation in 1,520 gene regions.[23] Subsequent pathway analysis suggested that these genes are involved in metabolic regulation and inflammation. In addition, rodent hypoxia studies have shown significant modification of circulating DNA and their association with glutamate metabolism and transport mechanisms.[21,22] Upon hypoxia exposure, these xenografted mice have showed less cellular organization compared to those who were not exposed to intermittent hypoxia, and systems biology analyses revealed an association with dysregulated molecular pathways and cancer progression.

Table 1—Epigenetic studies of hypoxia or OSA in humans.

Study	Number of Participants	Specimen	Method	Altered Mechanism	Key Observations With Hypoxia or OSA	Other Clinical Disorders With These Target Genes
Kim et al, 2012[17]	Control = 31, OSA = 47	Blood	Quantitative PCR	Inflammation	Hypermethylation of *FOXP3* and *IRF1* in children; high CRP levels in children; multiple regression models suggest AHI was independently associated with *FOXP3* methylation levels	Atherosclerosis, acute coronary syndrome, cancer
Kheirandish-Gozal et al, 2013[6]	Control = 35, OSA = 36	Blood	Pyrosequencing	Cardiovascular dysfunction	Hypermethylation levels at 171 positioned CpG sites of *eNOS*, and low mRNA expression; endothelial dysfunction, higher diastolic BP, increased serum lipids	Diabetes, obesity, migraine, hypertension
Chen et al, 2015[13]	Control = 8, OSA = 10	Visceral fat	Microarray	Metabolism, inflammation	Hypermethylation of promoter regions of *ACTA1, HDAC2,* and *SUMO1;* proteolysis	Congenital myopathy, orofacial cleft
Chen et al, 2016[12]	Control = 15, OSA = 6	Blood	Microarray	Inflammation, cardiovascular dysfunction	Differential methylation of *IL1R2, AR, NPR2,* and *SP140;* increased vasoconstriction, higher BP, increased catabolism	Acromesomelic dysplasia, Maroteaux type, epiphyseal chondrodysplasia, miura type
Cortese et al, 2016[18]	OSA = 15	Blood	Microarray	Inflammation	Increased methylation at peroxisome proliferation-activated receptors including *ABCA1, ABCG1,* CD36, *FABP4, HMOX*, *NOS2*, *PEPCK*, and *ADIPOQ*	Tangier disease, familial hypoalphalipoproteinemia, platelet glycoprotein IV deficiency, malaria, cancer, adiponectin deficiency
Sanz-Rubio et al, 2020[19]	Control = 38, OSA = 90	Blood	PCR, quantitative PCR	—	Unaltered methylation and gene expression of *FOXP3* in adults with OSA	Diabetes, atherosclerosis, acute coronary syndrome

ABCA1 = ATP binding cassette subfamily A member 1, *ABCG1* = ATP binding cassette subfamily G member 1, *ACTA1* = actin alpha 1, *ADIPOQ* = adiponectin, C1Q, and collagen domain containing, AHI = apnea-hypopnea index, *AR* = androgen receptor, BP = blood pressure, CD36 = CD36 molecule, CpG = cytosine and guanine separated by 1 phosphate group, CRP = C-reactive protein, *eNOS* = endothelial nitric oxide synthase, *FABP4* = fatty acid binding protein 4, *FOXP3* = forkhead box P3, *HDAC2* = histone deacetylase 2, *HMOX* = heme oxygenase, *IL1R2* = interleukin-1 receptor type 2, *IRF1* = interferon regulatory factor 1, *NOS2* = nitric oxide synthase 2, *NPR2* = natriuretic peptide receptor 2, OSA = obstructive sleep apnea, PCR = polymerase chain reaction, *PEPCK* = phosphoenolpyruvate carboxykinase, *SP140* = SP140 nuclear body protein, *SUMO1* = small ubiquitin-related modifier 1.

Inflammation

A majority (8/12) of OSA epigenetic studies showed hypermethylation of genes associated with inflammation. Studies in humans with OSA suggested that differential gene expression of actin alpha 1 could play a significant role in the regulation of adipocyte inflammation.[13] Hypermethylated interferon regulatory factor 1 and interleukin-1 receptor type 2 could potentially serve as biomarkers for OSA-associated inflammation.[12,17] Although forkhead box P3 hypermethylation has been suggested as a potential biomarker for OSA-associated inflammation in pediatric patients,[17] a recent study showed unaltered methylation and expression of forkhead box P3 in adult patients with OSA.[19] Therefore, the role of forkhead box P3 in OSA-associated inflammation is unclear.

Similar to human studies, epigenetic modifications have been identified in inflammatory pathways using animal models of OSA. An increase in the number of aortic macrophages was observed in mice exposed to chronic intermittent hypoxic conditions, and the transcription of several genes in these mice was altered to a proinflammatory state.[25] Nanduri and colleagues[20] discovered elevated reactive oxygen species and downregulation of antioxidant enzyme gene expression. Chronic intermittent hypoxia increased the DNA methylation of the antioxidant enzyme genes superoxide dismutase 1 and 2, thioredoxin reductase 2, and peroxiredoxin 4. Interestingly, these changes were

Table 2—Epigenetic studies of hypoxia or OSA in animal models.

Study	Number of Animals	Specimen	Method	Altered Mechanism	Key Observations with Hypoxia or OSA	Other Clinical Disorders with These Target Genes
Cortese et al, 2015[22]	Control = 3, IH group = 3	Blood	Microarray	Metabolism	Higher cirDNA modification	Diabetes, stroke, cancer, fetal disorders, sickle cell disease
Cortese et al, 2015[21]	Control = 3, IH group = 3	Blood	Microarray	—	Differential cirDNA modification with high variability regions especially in chromosomes 7,13,14, and ×.	—
Nanduri et al, 2017[20]	Control experiments = 3, IH experiments = 3	Blood	PCR	Inflammation, cardiovascular dysfunction	Long-term intermittent hypoxia increases DNA methylation and persistent downregulation of genes encoding for antioxidant enzymes including *Sod1, Sod2, Txnrd2,* and *Prdx4;* elevated reactive oxygen species; increased BP, plasma norepinephrine levels, persistent carotid body chemosensory reflex	Amyotrophic lateral sclerosis, type 1; diabetes, cardiomyopathy, neurodevelopmental disorders
Cortese et al, 2017[25]	Control = 8, IH group = 8	Aorta macrophages	MicroChip analysis	Inflammation, cardiovascular dysfunction	Activation of proatherogenic pathways involving histone modifications with inflammation and oxidative stress; vascular dysfunction; increased intima-media thickness	Atherosclerosis
Khalyfa et al, 2017[23]	Control = 8, IH group = 8	Visceral fat	Methylated DNA immunoprecipitation	Metabolism, inflammation, cardiovascular dysfunction	Differential methylation; hyper DNA methylation with late gestational intermittent hypoxia mainly associated with molecules of inflammatory responses and signaling pathways like IL-4, IL-12, ILK, and NFκB; late-gestation intermittent hypoxia leads to increased body weight, food intake, insulin resistance, and higher cholesterol	Immunodeficiency, cardiomyopathy, osteomyelitis
Badran et al, 2019[24]	Control = 4–5, IH group = 4–5	PVAT mature adipocytes	Pyrosequencing	Metabolism, inflammation, cardiovascular dysfunction	Increased body weight, food consumption, oxidative stress, and endothelial dysfunction in adult male offspring; increased TNF levels in plasma upon gestational intermittent hypoxia; hypermethylation of adiponectin in male pups	Vascular dementia, Alzheimer disease, psoriatic arthritis, Crohn disease, rheumatoid arthritis

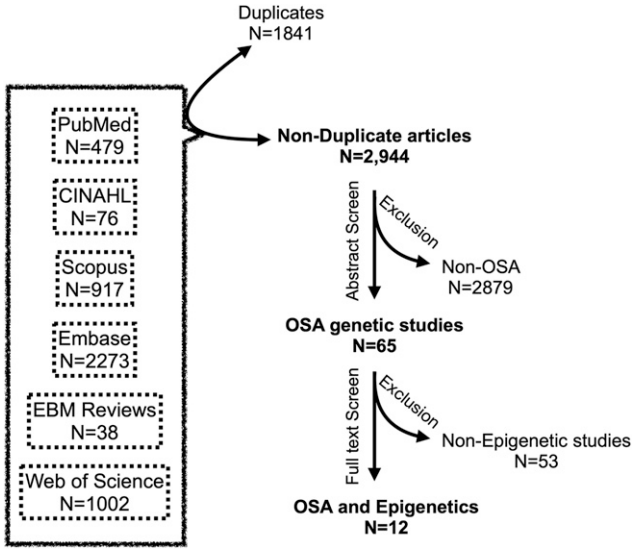
BP = blood pressure, cirDNA = circulating DNA, IH = intermittent hypoxia, IL-4 = interleukin-4, IL-12 = interleukin-12, ILK = integrin linked kinase, NFκB = Nuclear Factor Kappa B, OSA = obstructive sleep apnea, PCR = polymerase chain reaction, *Prdx4* = peroxiredoxin 4, PVAT = perivascular adipose tissue, *Sod1* = superoxide dismutase 1, *Sod2* = superoxide dismutase 2, *Txnrd2* = thioredoxin reductase 2, TNF = tumor necrosis factor.

preventable. When these mice were given decitabine, an inhibitor of DNA methylation, the antioxidant enzyme gene expression for superoxide dismutase 1, superoxide dismutase 2, thioredoxin reductase 2, and peroxiredoxin 4 was restored and the reactive oxygen species levels returned to baseline.[20] Furthermore, hypermethylated peroxisome proliferation-activated receptors, receptors associated with immune responses, have been identified under hypoxic conditions.[18]

Cardiovascular dysfunction

Many genes with OSA-dependent expression were associated with cardiovascular dysfunction in OSA. In human patients, children with OSA had hypermethylated sites of cytosine and guanine separated by 1 phosphate group at the endothelial nitric oxide synthase gene promoter and 6 adjacent sites of cytosine and guanine separated by 1 phosphate group. As a result, the endothelial nitric oxide synthase mRNA levels were significantly

Figure 1—PRISMA diagram of systematic review for epigenetics and obstructive sleep apnea.



Overview of the number of studies that were included or excluded in each phase of the study selection procedure, as outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. OSA = obstructive sleep apnea.

reduced, which likely accounts for the impaired peripheral vascular function observed in pediatric patients with OSA.[6] Pediatric patients with OSA also have higher diastolic blood pressure (BP) and greater serum lipids compared to healthy control patients.[6]

In adults, there was an association between an increased oxygen desaturation index and excessive daytime sleepiness with differential methylation in the promoter regions for the interleukin-1 receptor type 2, androgen receptor, natriuretic peptide receptor 2, and SP140 nuclear body protein genes.[12] This finding suggests that epigenetic programming may play a role in excessive daytime sleepiness in patients with OSA. Hypomethylation occurred in 5 differentially methylated loci involved with the natriuretic peptide receptor 2 pathway, which may play a crucial role in the development of the excessive daytime sleepiness phenotype characterized by increased catabolism, higher BP, and increased vasoconstriction.[12]

In animal models, late gestational intermittent hypoxia led to increased body weight, food intake, visceral and subcutaneous mass, insulin resistance, and cholesterol.[23] Interestingly, the effects of maternal late gestational intermittent hypoxia were only seen in male mice but not in female mice. Similarly, male mice had altered macrophage populations and changes in DNA methylation of the visceral white adipose tissue not seen in female mice. Furthermore, a recent study suggested that gestational intermittent hypoxia causes endothelial dysfunction and hypermethylation of the adiponectin gene promoter in adult male offspring.[24] These observations have been correlated with increased body weight and inflammatory responses in male offspring.

When exposed to chronic intermittent hypoxia, rodents indicated vascular dysfunction with increased intima-media thickness and disruption in the integrity of the elastic laminae.[25] Moreover, there was an increase in the number of macrophages observed in the aorta with differential gene transcription to a proatherogenic state. In addition, chronic intermittent hypoxia leads to elevated BP, elevated norepinephrine levels, and persistent activation of the carotid body chemosensory reflex. However, when mice were treated with decitabine, an inhibitor of DNA methylation, their cardiorespiratory functions (BP) normalized, norepinephrine levels stabilized, and carotid body sensory activity improved.[20]

DISCUSSION

Polysomnograms are the current gold-standard instruments for the diagnosis of OSA,[26] but they are time-consuming, costly, and not always easily accessible.[27] These factors are especially challenging in the pediatric population, given the limited number of pediatric sleep centers.[27,28] Fewer than 10% of children with sleep-disordered breathing undergo a polysomnogram before surgical intervention with an adenotonsillectomy.[29] It is imperative to identify a better way to diagnose and monitor OSA severity given the significant burden it imposes on the health care system. In 2015, an estimated $12.4 billion was spent on diagnosing and treating OSA.[3] However, because of the large number of patients who are undiagnosed, with a prevalence of greater than 30% of adults between ages 30 and 69 years,[2] the yearly cost for undiagnosed OSA and the associated morbidities is estimated to be $150 billion. These facts highlight the importance of identifying reliable, inexpensive, and readily available biomarkers, especially for those patients with less access to medical care.

Genomic markers are currently being used as a screening tool in other diseases including gastrointestinal cancer,[30] ovarian cancer,[31] eosinophilic esophagitis,[32] Alzheimer disease,[33] asthma,[34] and inflammatory bowel disease.[35] Building on this development, epigenetic modifications have been used to identify changes in other conditions such as amyotrophic lateral sclerosis,[36] cancer,[15] prenatal tobacco smoke exposure,[37] and food allergies.[38] These same principles could be used to study OSA.

Recent studies have begun to search for biomarkers of OSA that could serve as diagnostic instruments. One potential noninvasive method is screening urine. Becker and colleagues[39] performed protein analysis of urine from children with OSA and healthy control patients. They identified 192 candidate biomarkers of pediatric OSA. In a similar study for biomarkers of OSA among urine samples from children, 2 distinct proteins from the OSA group were identified.[40] Breath condensate has been evaluated as another noninvasive sample. Interleukin-10 is an anti-inflammatory cytokine that regulates normal sleep patterns, and interleukin-10 levels from breath condensate have corresponded with the severity of OSA.[41–44] Two studies investigated gene expression profiling from blood in patients with OSA, 1 in adults and the other in children. Adult patients with OSA were found to have sleep-associated upregulation of oxidative stress response genes, such as superoxide dismutase 2 and

catalase, and downregulation of superoxide dismutase 1.[11] Among pediatric patients, numerous differentially expressed genes were identified in those with OSA, including those involved in inflammatory pathways.[45] In studies screening the entire genome for OSA and obesity, 2 candidate regions on chromosomes were identified among patients of European ancestry, suggesting that there is an association between OSA severity when controlling for body mass index.[46] Similar findings have been identified among Black patients with OSA.[47] Finally, telomere shortening, a consequence seen in multiple systemic diseases, may provide another screening avenue. Patients with OSA were found to have significantly shortened telomere length in peripheral blood leukocytes.[48,49] However, this hypothesis is somewhat controversial; for example, Polonis et al[50] found that moderate to severe OSA was associated with telomere lengthening in genomic DNA from peripheral blood samples.

Our systematic review revealed limited research on epigenetic modification and associated changes in gene expression in OSA. Despite this result, several studies showed unique changes in methylation among patients with OSA compared to healthy control patients. When assessed, the genes with epigenetic changes corresponded with differences in protein expression in the pathways most frequently associated with metabolism and inflammation.[12,13,17,20,23] Similar information has been gleaned from analyses of whole-gene expression. For example, up- and downregulation of specific genes have been identified in patients with OSA, findings that normalize after treatment with continuous positive airway pressure.[51] The expression of specific genes in whole blood has even predicted apnea-hypopnea index scores.[52] Tissue-specific genetic analysis of soft palate tissue in patients with OSA showed differential expression of genes related to the pathophysiology of OSA.[53] This supports the important role that genetic markers could provide in both disease screening and monitoring response to treatment.

By looking at epigenetic modification, disease presence and disease severity may be able to be identified and monitored. Chen, Yang, and colleagues[13] found that differentially expressed genes could significantly distinguish OSA samples from normal control samples, suggesting that this characteristic could provide a future screening mechanism for OSA. Along these lines, changes in *FOXP3* have been independently associated with the apnea-hypopnea index, showing how epigenetic modification may be able to serve as a marker of disease severity.[17] Genomic markers could provide a novel, inexpensive, efficient, and widely accessible tool to diagnose and screen for OSA. This tool would facilitate ease of diagnosis among patients, which would be especially important for underserved patient populations.

Studies of epigenetic modification may even provide new options for treatment. When Nanduri and colleagues[20] treated mice with an inhibitor of DNA methylation, all the negative effects of chronic intermittent hypoxia resolved, including increased BP, norepinephrine levels, and carotid body sensory activity. Because epigenetic alterations are potentially reversible, they are currently being investigated in other diseases.[54] In fact, there are currently 2 DNA methyltransferase inhibitors that are approved by the U.S. Food & Drug Administration for the treatment of acute myeloid leukemia and myelodysplastic syndrome.[55] Epigenetic drugs have also been shown to help reduce infarct size after stroke,[56] improve memory formation and retention with promising results for Alzheimer disease,[57,58] and improve recovery after myocardial infarction.[59] Targeting these epigenetic modifications may reduce or reverse the complications associated with OSA.

Limitations

There are several limitations to our study worth discussing. During our literature search, we excluded those studies not written in the English language or without available full texts, potentially resulting in a selection bias. It is possible that our search terms did not allow for the inclusion of all studies associated with epigenetic modification in models of or patients with OSA; however, an exhaustive search was performed in 6 of the most commonly used academic databases, including PubMed, CINAHL, Scopus, Embase, EBM Reviews, and Web of Science. Overall, there are a limited number of studies directly addressing epigenetic modification in OSA. In addition, these limited studies are conducted across heterogeneous populations and in different animal models. However, it is clear that future work should focus on the potential role of epigenetic modification in the diagnosis and treatment of OSA and those associated medical comorbidities.

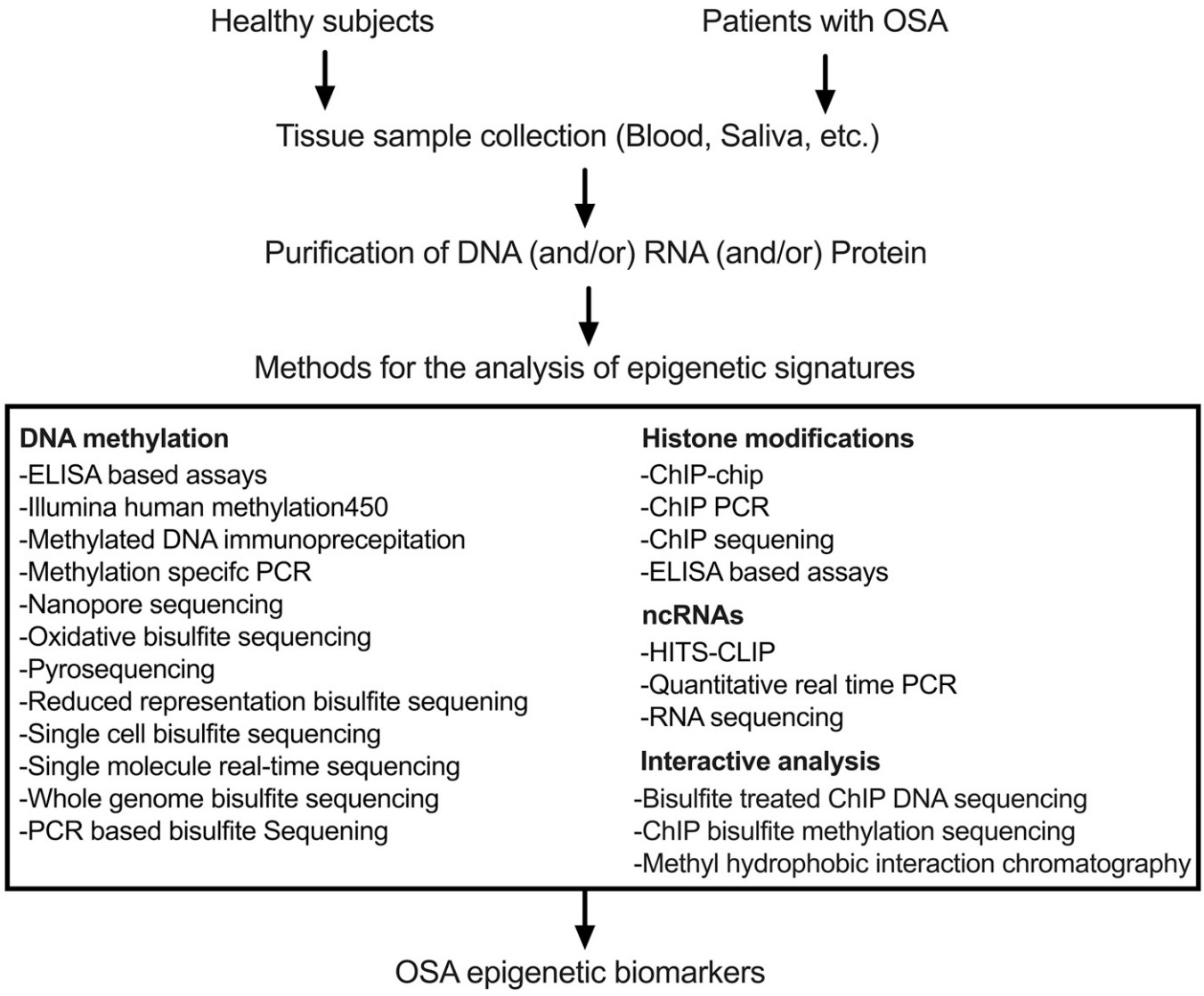
Future directions

Gene expression patterns are commonly used molecular biomarkers for disease diagnosis, prognosis, and therapy for several health conditions including the modern global pandemic, COVID-19.[60] However, the recent development in biomarker technology suggests that epigenetic modifications (DNA methylation, histone modifications, and changes in noncoding RNA) could potentially serve as next-generation biomarkers mainly because of their ability to buffer with environmental variation, population diversity, and disease state.[61] By considering the advantage of epigenetic plasticity with a disease condition, health care professionals may find that epigenetic signatures may serve as next-generation diagnostic tools for a highly prevalent disorder like OSA. However, there is a gap in understanding the use of epigenetic signatures for clinical diagnostics.

Here, we describe how epigenetic biomarkers may be used in next-generation diagnostics. So far, the studies that have evaluated epigenetic signatures in patients with OSA or animal models representing OSA have used an array of methods (eg, quantitative polymerase chain reaction, polymerase chain reaction, microarray, microchip, methylated DNA immunoprecipitation, and pyrosequencing) for rodent and human samples. However, the use of heterogeneous study populations has made these methods very difficult to compare equally. Based on the variability with techniques and analyses, these results cannot be applied to all patients with OSA. Therefore, it is important to discern how epigenetic modifications present in different phenotypes and how different investigative methods affect findings. For these reasons, large prospective studies that use standardized techniques among homogeneous populations are necessary.

There are a few important factors to be considered for testing OSA epigenetic markers, including (1) selection of patients, (2) tissue type, and (3) technology and data analysis. Unlike the

Figure 2—A prospective study design for the development of OSA-specific epigenetic biomarkers.



To develop OSA-specific epigenetic biomarkers, DNA, RNA, or proteins should be purified from tissue samples of both healthy patients and patients with OSA. The listed epigenetic methods may be used for understanding the OSA-specific epigenetic signatures. ChIP = chromatin immunoprecipitation, ELISA = enzyme-linked immunosorbent assay, HITS = high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation, OSA = obstructive sleep apnea, PCR = polymerase chain reaction.

genome, the epigenome is not stable and varies with age, sex, and environmental conditions. A precise description of patient demographics and clearly defined pre-existing health conditions are important for epigenetic association studies. The control group must be precisely selected based on the diagnostic criteria. Another key factor for epigenetic studies is tissue selection. Epigenome markers are tissue-specific and differ across tissues and organs.[61] So far, the majority of human epigenome studies have used body fluids (ie, saliva, blood, and urine). However, the selection of inappropriate tissues may raise false-positive results. Therefore, tissue selection must be accurately defined with pathogenesis specific to those patients with OSA. Several methods have been developed to understand epigenetic modifications including pyrosequencing, bisulfite sequencing, methylation arrays, and quantitative reverse transcription polymerase chain reaction.[62] Among these methods, the candidate gene approach methods, like quantitative reverse transcription polymerase chain reaction, are cost-effective for OSA diagnostics in a larger population. However, this approach misses the vast majority of information from the entire genome. Although genome-level studies are not economical for diagnostics in a larger population, focused, systematic epigenome studies in animal models and humans may provide strength for establishing cost-effective epigenetic biomarkers for use in patients with OSA. Here, we offer a study design for developing OSA biomarkers with a list of available epigenetic methods (**Figure 2**).

CONCLUSIONS

We found that there are differential changes in epigenetic modification and gene expression in patients with OSA. These findings highlight the potential significance of epigenetic studies for use in diagnosing OSA. More important, they may also further our understanding of the pathophysiologic progression of diseases commonly associated with this complex disorder.

ABBREVIATIONS

BP, blood pressure
OSA, obstructive sleep apnea

REFERENCES

1. Marcus CL, Brooks LJ, Draper KA, et al. American Academy of Pediatrics. Diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics.* 2012;130(3):e714–e755.
2. Benjafield AV, Ayas NT, Eastwood PR, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. *Lancet Respir Med.* 2019;7(8):687–698.
3. Frost and Sullivan. Hidden health crisis costing America billions: underdiagnosing and undertreating obstructive sleep apnea draining healthcare system. Report for the American Academy of Sleep Medicine. Published 2016. https://aasm.org/resources/pdf/sleep-apnea-economic-crisis.pdf. Accessed July 23, 2021.
4. Parish JM, Somers VK. Obstructive sleep apnea and cardiovascular disease. *Mayo Clin Proc.* 2004;79(8):1036–1046.
5. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest.* 1995;96(4):1897–1904.
6. Kheirandish-Gozal L, Khalyfa A, Gozal D, Bhattacharjee R, Wang Y. Endothelial dysfunction in children with obstructive sleep apnea is associated with epigenetic changes in the eNOS gene. *Chest.* 2013;143(4):971–977.
7. Bhattacharjee R, Kim J, Alotaibi WH, Kheirandish-Gozal L, Capdevila OS, Gozal D. Endothelial dysfunction in children without hypertension: potential contributions of obesity and obstructive sleep apnea. *Chest.* 2012;141(3):682–691.
8. Gozal D, Serpero LD, Sans Capdevila O, Kheirandish-Gozal L. Systemic inflammation in non-obese children with obstructive sleep apnea. *Sleep Med.* 2008;9(3):254–259.
9. Tauman R, Lavie L, Greenfeld M, Sivan Y. Oxidative stress in children with obstructive sleep apnea syndrome. *J Clin Sleep Med.* 2014;10(6):677–681.
10. Jurado-Gamez B, Gomez-Chaparro JL, Muñoz-Calero M, et al. Serum proteomic changes in adults with obstructive sleep apnoea. *J Sleep Res.* 2012;21(2):139–146.
11. Hoffmann MS, Singh P, Wolk R, Romero-Corral A, Raghavakaimal S, Somers VK. Microarray studies of genomic oxidative stress and cell cycle responses in obstructive sleep apnea. *Antioxid Redox Signal.* 2007;9(6):661–669.
12. Chen Y-C, Chen T-W, Su M-C, et al. Whole genome DNA methylation analysis of obstructive sleep apnea: IL1R2, NPR2, AR, SP140 methylation and clinical phenotype. *Sleep.* 2016;39(4):743–755.
13. Chen JH, Yang R, Wang YP, Zhang W. Expression data analysis to identify key target genes in visceral fat tissue associated with obstructive sleep apnea. *Eur Rev Med Pharmacol Sci.* 2015;19(22):4293–4299.
14. Cheng Y, He C, Wang M, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther.* 2019;4(1):62.
15. Joyce BT, Gao T, Zheng Y, et al. Prospective changes in global DNA methylation and cancer incidence and mortality. *Br J Cancer.* 2016;115(4):465–472.
16. Hwang J-Y, Aromolaran KA, Zukin RS. Author correction: the emerging field of epigenetics in neurodegeneration and neuroprotection. *Nat Rev Neurosci.* 2018;19(12):771.
17. Kim J, Bhattacharjee R, Khalyfa A, et al. DNA methylation in inflammatory genes among children with obstructive sleep apnea. *Am J Respir Crit Care Med.* 2012;185(3):330–338.
18. Cortese R, Zhang C, Bao R, et al. DNA methylation profiling of blood monocytes in patients with obesity hypoventilation syndrome: effect of positive airway pressure treatment. *Chest.* 2016;150(1):91–101.
19. Sanz-Rubio D, Sanz A, Varona L, et al. Forkhead box P3 methylation and expression in men with obstructive sleep apnea. *Int J Mol Sci.* 2020;21(6):2233.
20. Nanduri J, Peng Y-J, Wang N, et al. Epigenetic regulation of redox state mediates persistent cardiorespiratory abnormalities after long-term intermittent hypoxia. *J Physiol.* 2017;595(1):63–77.
21. Cortese R, Almendros I, Wang Y, Gozal D. Microarray-based analysis of plasma cirDNA epigenetic modification profiling in xenografted mice exposed to intermittent hypoxia. *Genom Data.* 2015;5:17–20.
22. Cortese R, Almendros I, Wang Y, Gozal D. Tumor circulating DNA profiling in xenografted mice exposed to intermittent hypoxia. *Oncotarget.* 2015;6(1):556–569.
23. Khalyfa A, Cortese R, Qiao Z, et al. Late gestational intermittent hypoxia induces metabolic and epigenetic changes in male adult offspring mice. *J Physiol.* 2017;595(8):2551–2568.
24. Badran M, Yassin BA, Lin DTS, Kobor MS, Ayas N, Laher I. Gestational intermittent hypoxia induces endothelial dysfunction, reduces perivascular adiponectin and causes epigenetic changes in adult male offspring. *J Physiol.* 2019;597(22):5349–5364.
25. Cortese R, Gileles-Hillel A, Khalyfa A, et al. Aorta macrophage inflammatory and epigenetic changes in a murine model of obstructive sleep apnea: potential role of CD36. *Sci Rep.* 2017;7(1):43648.
26. Farber JM. Clinical practice guideline: diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics.* 2002;110(6):1255–1257.
27. Portier F, Portmann A, Czernichow P, et al. Evaluation of home versus laboratory polysomnography in the diagnosis of sleep apnea syndrome. *Am J Respir Crit Care Med.* 2000;162(3 Pt 1):814–818.
28. Katz SL, Witmans M, Barrowman N, et al. Paediatric sleep resources in Canada: the scope of the problem. *Paediatr Child Health.* 2014;19(7):367–372.
29. Mitchell RB, Pereira KD, Friedman NR. Sleep-disordered breathing in children: survey of current practice. *Laryngoscope.* 2006;116(6):956–958.
30. Chen H-M, Fang J-Y. Epigenetic biomarkers for the early detection of gastrointestinal cancer. *Gastrointest Tumors.* 2014;1(4):201–208.
31. Su H-Y, Lai H-C, Lin Y-W, Chou Y-C, Liu C-Y, Yu M-H. An epigenetic marker panel for screening and prognostic prediction of ovarian cancer. *Int J Cancer.* 2009;124(2):387–393.
32. Sherrill JD, Rothenberg ME. Genetic and epigenetic underpinnings of eosinophilic esophagitis. *Gastroenterol Clin North Am.* 2014;43(2):269–280.
33. Loring JF, Wen X, Lee JM, Seilhamer J, Somogyi R. A gene expression profile of Alzheimer's disease. *DNA Cell Biol.* 2001;20(11):683–695.
34. Howrylak JA, Moll M, Weiss ST, Raby BA, Wu W, Xing EP. Gene expression profiling of asthma phenotypes demonstrates molecular signatures of atopy and asthma control. *J Allergy Clin Immunol.* 2016;137(5):1390–1397.e6.
35. van Beeland Granlund A, Flatberg A, Østvik AE, et al. Whole genome gene expression meta-analysis of inflammatory bowel disease colon mucosa demonstrates lack of major differences between Crohn's disease and ulcerative colitis. *PLoS One.* 2013;8(2):e56818.
36. Tremolizzo L, Messina P, Conti E, et a; EURALS Consortium. Whole-blood global DNA methylation is increased in amyotrophic lateral sclerosis independently of age of onset. *Amyotroph Lateral Scler Frontotemporal Degener.* 2014;15(1–2):98–105.
37. Breton CV, Byun H-M, Wenten M, Pan F, Yang A, Gilliland FD. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *Am J Respir Crit Care Med.* 2009;180(5):462–467.

38. Lee KH, Song Y, O'Sullivan M, Pereira G, Loh R, Zhang GB. The implications of DNA methylation on food allergy. *Int Arch Allergy Immunol.* 2017;173(4): 183–192.
39. Becker L, Kheirandish-Gozal L, Peris E, Schoenfelt KQ, Gozal D. Contextualised urinary biomarker analysis facilitates diagnosis of paediatric obstructive sleep apnoea. *Sleep Med.* 2014;15(5):541–549.
40. Snow A, Gozal D, Valdes R Jr, Jortani SA. Urinary proteins for the diagnosis of obstructive sleep apnea syndrome. *Methods Mol Biol.* 2010;641:223–241.
41. Özdaş S, Özdaş T, Acar M, et al. Association of interleukin-10 gene promoter polymorphisms with obstructive sleep apnea. *Sleep Breath.* 2016;20(2):855–866.
42. De Luca Canto G, Pachêco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Biomarkers associated with obstructive sleep apnea: a scoping review. *Sleep Med Rev.* 2015;23:28–45.
43. De Luca Canto G, Pachêco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Diagnostic capability of biological markers in assessment of obstructive sleep apnea: a systematic review and meta-analysis. *J Clin Sleep Med.* 2015; 11(1):27–36.
44. Li Y, Chongsuvivatwong V, Geater A, Liu A. Exhaled breath condensate cytokine level as a diagnostic tool for obstructive sleep apnea syndrome. *Sleep Med.* 2009; 10(1):95–103.
45. Khalyfa A, Capdevila OS, Buazza MO, Serpero LD, Kheirandish-Gozal L, Gozal D. Genome-wide gene expression profiling in children with non-obese obstructive sleep apnea. *Sleep Med.* 2009;10(1):75–86.
46. Palmer LJ, Buxbaum SG, Larkin E, et al. A whole-genome scan for obstructive sleep apnea and obesity. *Am J Hum Genet.* 2003;72(2):340–350.
47. Palmer LJ, Buxbaum SG, Larkin EK, et al. Whole genome scan for obstructive sleep apnea and obesity in African-American families. *Am J Respir Crit Care Med.* 2004;169(12):1314–1321.
48. Kim KS, Kwak JW, Lim SJ, Park YK, Yang HS, Kim HJ. Oxidative stress-induced telomere length shortening of circulating leukocyte in patients with obstructive sleep apnea. *Aging Dis.* 2016;7(5):604–613.
49. Choi K-M, Thomas RJ, Yoon DW, Lee SK, Baik I, Shin C. Interaction between obstructive sleep apnea and shortened telomere length on brain white matter abnormality. *Sleep.* 2016;39(9):1639–1645.
50. Polonis K, Somers VK, Becari C, et al. Moderate-to-severe obstructive sleep apnea is associated with telomere lengthening. *Am J Physiol Heart Circ Physiol.* 2017;313(5):H1022–H1030.
51. Chen Y-C, Chen K-D, Su M-C, et al. Genome-wide gene expression array identifies novel genes related to disease severity and excessive daytime sleepiness in patients with obstructive sleep apnea. *PLoS One.* 2017;12(5): e0176575.
52. Perry JC, Guindalini C, Bittencourt L, Garbuio S, Mazzotti DR, Tufik S. Whole blood hypoxia-related gene expression reveals novel pathways to obstructive sleep apnea in humans. *Respir Physiol Neurobiol.* 2013;189(3):649–654.
53. Song CM, Lee CH, Rhee C-S, Min Y-G, Kim J-W. Analysis of genetic expression in the soft palate of patients with obstructive sleep apnea. *Acta Otolaryngol.* 2012; 132(Suppl 1):S63–S68.
54. Heerboth S, Lapinska K, Snyder N, Leary M, Rollinson S, Sarkar S. Use of epigenetic drugs in disease: an overview. *Genet Epigenet.* 2014;6:9–19.
55. Gnyszka A, Jastrzebski Z, Flis S. DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Res.* 2013;33(8): 2989–2996.
56. Ren M, Leng Y, Jeong M, Leeds PR, Chuang D-M. Valproic acid reduces brain damage induced by transient focal cerebral ischemia in rats: potential roles of histone deacetylase inhibition and heat shock protein induction. *J Neurochem.* 2004;89(6):1358–1367.
57. Guan J-S, Haggarty SJ, Giacometti E, et al. HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature.* 2009;459(7243):55–60.
58. Kilgore M, Miller CA, Fass DM, et al. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology.* 2010;35(4):870–880.
59. Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. *Nat Rev Cardiol.* 2010;7(9):510–519.
60. Carter LJ, Garner LV, Smoot JW, et al. Assay techniques and test development for COVID-19 diagnosis. *ACS Cent Sci.* 2020;6(5):591–605.
61. Riancho J, Del Real A, Riancho JA. How to interpret epigenetic association studies: a guide for clinicians. *Bonekey Rep.* 2016;5:797.
62. Li Y. Modern epigenetics methods in biological research. *Methods.* 2021;187: 104–113. 32645449

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