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Proteomic biomarkers of cognitive impairment in obstructive sleep apnea syndrome

Chitra Lal[1] · Gary Hardiman[2] · Suchit Kumbhare[1,3] · Charlie Strange[1]

Abstract

Purpose There are currently no biomarkers that are associated with cognitive impairment (CI) in patients with obstructive sleep apnea syndrome (OSAS). This pilot study performed an exploratory plasma proteomic analysis to discover potential biomarkers and explore proteomic pathways that differentiate OSAS subjects with and without CI.

Methods Participants were selected from a cohort of women within 5 years of menopause not on hormone replacement therapy between the ages of 45–60 years. The Berlin questionnaire was used to select OSAS participants who then completed the MCFSI (Mail-In Cognitive Function Screening Instrument) to measure cognition. Six subjects with the highest MCFSI scores (≥ 5 denoting CI) were compared to six with normal scores. Proteomic analysis was done by Myriad RBM using a targeted ELISA for 254 serum proteins. Pathway analysis of differentially expressed proteins was performed using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) software.

Results Distinct proteomic signatures were seen in OSAS subjects with CI as compared to those without CI. Proteins including insulin, prostasin, angiopoietin-1, plasminogen activator inhibitor 1, and interleukin-1 beta were overexpressed in OSAS subjects with CI. Proteins underexpressed in CI participants included cathepsin B, ceruloplasmin, and adiponectin. Pathway analysis revealed prominence of insulin-regulated vascular disease biomarkers.

Conclusions Proteomic biomarkers in participants with cognitive impairment suggest roles for insulin, and vascular signaling pathways, some of which are similar to findings in Alzheimer's disease. A better understanding of the pathogenic mechanisms of CI in OSAS will help focus clinical trials needed in this patient population.

Keywords Cognitive impairment · Proteomics · Biomarkers · Obstructive sleep apnea syndrome · Menopause

✉ Chitra Lal
lalch@musc.edu

Gary Hardiman
hardiman@musc.edu

Suchit Kumbhare
kumbhare@musc.edu

Charlie Strange
strangec@musc.edu

[1] Pulmonary, Critical Care, Allergy and Sleep Medicine, Medical University of South Carolina, 96 Jonathan Lucas Street, CSB Suite 816, MSC 630, Charleston, SC 29425, USA

[2] MUSC Bioinformatics, Center for Genomics Medicine, Medical University of South Carolina, Charleston, SC, USA

[3] Medical University of South Carolina, Charleston, SC, USA

Abbreviations

OSAS	Obstructive sleep apnea syndrome
CI	Cognitive impairment
SCOR	Specialized center of research
HRT	Hormone replacement therapy
MCFSI	Mail-In Cognitive Function Screening Instrument
AUC	Area under the curve
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
BMI	Body mass index
AD	Alzheimer's disease
IL1B	Interleukin-1 beta
ELAM-1	Endothelial-leukocyte adhesion molecule 1
CTSB	Cathepsin B
APP	Amyloid precursor protein

Introduction

Obstructive sleep apnea syndrome (OSAS) is a common disorder characterized by a decrease or cessation of airflow due to upper airway obstruction. It has a prevalence of 6% in women and 13% in men in the 30–70 years age group [1]. There is emerging evidence that cognitive deficits are associated with OSAS [2, 3]. Cognitive domains most commonly affected in patients with OSAS include vigilance, memory, and executive function [2, 4, 5]. Some studies have found a correlation between the intermittent, episodic hypoxemia of OSAS and cognitive impairment (CI) [6], while others have found that neuro-structural brain changes occur in OSAS [7] and these are associated with cognitive deficits. Some of these changes can be reversed with continuous positive airway pressure treatment of OSAS [7]. However, the pathophysiologic mechanisms underlying the CI seen in OSAS patients are still unclear. There are currently no biomarkers that accurately predict cognitive decline or worse cognitive outcomes in patients with OSAS. Identification of such biomarkers may help to further delineate the pathogenesis of CI in OSAS and also produce targets for diagnostic use or future therapeutic interventions.

We hypothesized that subjects with OSAS and CI would exhibit different plasma proteomic signatures as compared to subjects with OSAS and no CI. To this aim, we performed a pilot study with an exploratory large-scale proteomic analysis of plasma to see if there were any differences in proteomic pathways between these 2 groups of subjects. Our long-term goal is to define new OSAS clinical phenotypes associated with proteomic biomarkers that explain some of the clinical diversity, and inform treatment targets for OSAS comorbidities.

Studies which focus on all patients with OSAS may miss subtle associations between OSAS and CI [8]. However, focusing on specific populations such as early postmenopausal women as in this study may help to define proteomic correlations that would advance the science of associations between CI and OSAS which may occur in some disease phenotypes.

Materials and methods

Subjects enrolled in the SCOR (Specialized Center of Research) study ($N = 254$) previously published by the authors [9] were invited to provide blood samples for this IRB approved study.

Participants included early postmenopausal women not on hormone replacement therapy (HRT) between the ages of 45 and 60 years, who were within 5 years of having achieved natural menopause and were determined to be at high risk of having OSAS as defined by the Berlin questionnaire.

Table 1 Components of the MCFSI questionnaire

Question	Patient response		
	Yes = 1	No = 0	Maybe = 0.5
1. Compared to one year ago, do you feel that your memory has declined substantially?			
2. Do others tell you that you tend to repeat questions over and over?			
3. Have you been misplacing things more often?			
4. Do you find that lately you are relying more on written reminders?			
5. Do you need more help from others to remember appointments, family occasions or holidays?			
6. Do you have more trouble recalling names, finding the right word or completing sentences?			
7. Do you have more trouble driving?			
8. Compared to one year ago, do you have more difficulty managing money?			
9. Are you less involved in social activities?			
10. Has your work performance declined significantly compared to one year ago?			
11. Do you have more trouble following the news, or the plots of books, movies or TV shows, compared to one year ago?			
12. Are there any activities that are substantially more difficult for you now compared to one year ago?			
13. Are you more likely to become disoriented, or get lost, for example, when traveling to another city?			
14. Do you have more difficulty using household appliances?			
	Total Score		

Table 2 Underexpressed proteins in OSAS subjects with increasing MCFSI scores

Protein/abbreviation	Regression coefficient with MCFSI (R^2 value)	Regression p value	Categorical AUC	AUC p value
Leucine rich alpha-2 glycoprotein (LRG1)	0.608	0.001	0.944	0.002
Osteopontin (OPN)	0.59	0.002	1	< 0.0001
Adiponectin (ADIPOQ)	0.52	0.005	1	< 0.0001
Sclerostin (SOST)	0.5	0.006	1	< 0.0001
CEA antigen related cell adhesion molecule 6 (CEACAM6)	0.402	0.015	0.902	0.004
Insulin like growth factor binding protein 2 (IGFBP2)	0.403	0.015	0.944	0.003
Angiopoietin related protein 4 (ANGPTL4)	0.356	0.02	0.777	0.015
Pentraxin-3 (PTX3)	0.343	0.026	0.861	0.017
Cathepsin B (CTSB)	0.314	0.033	0.833	0.033
Ceruloplasmin (CP)	0.276	0.045	0.916	0.003
Myeloperoxidase (MPO)	0.267	0.049	0.791	0.056

OSAS obstructive sleep apnea syndrome, *MCFSI* Mail-In Cognitive Function Screening Instrument, *AUC* area under the curve

Menopause was defined by a history of no menstrual bleeding for ≥ 1 year.

Subjects were categorized as having CI vs. no CI by the MCFSI (Mail-In Cognitive Function Screening Instrument). We used MCFSI score ≥ 5 to categorize subjects as having CI and an MCFSI score < 5 as not having CI. MCFSI was developed for the Alzheimer's Disease Cooperative Study (ADCS) Prevention Instrument Project as an open-access survey to evaluate whether a screening tool could be used to trigger a diagnostic evaluation in large dementia prevention trials [10]. It is a short, self-administered test which measures the degree of self-perceived cognitive impairment with higher scores correlating with worse cognition. Correlation has been seen between the MCFSI total scores, the Mini-Mental Status Examination scores and the Clinical Dementia Rating Scores in healthy elderly individuals. We have adopted the MCFSI as an easily administered screening tool for CI in our OSAS population [9, 11, 12]. Permission was obtained from the author/journal to use the Berlin and MCFSI questionnaires. Components of the MCFSI questionnaire are shown in Table 1.

Additional data collected through a REDCap survey [13] included demographic data as well as data on risk factors for CI such as health care professional diagnosed hypertension, diabetes mellitus, stroke, heart disease, depression, and treatment for depression [3]. Subjects were also asked about a self-

Table 3 Overexpressed proteins in OSAS subjects with increasing MCFSI scores

Protein/abbreviation	Regression coefficient with MCFSI (R^2 value)	Regression p value	Categorical AUC	AUC p value
Prostasin (PRSS8)	0.604	0.001	0.916	0.002
Interleukin-1 beta (IL1B)	0.462	0.008	0.958	0.0005
Pigment epithelium derived factor (SERPINF1)	0.473	0.008	0.888	0.003
Plasminogen activator inhibitor 1 (SERPINE1)	0.434	0.011	0.93	0.002
Pulmonary and activation regulated chemokine (CCL18)	0.432	0.012	0.805	0.086
Insulin (INS)	0.553	0.013	0.888	0.029
E-selectin (SELE)	0.41	0.014	0.888	0.011
Angiopoietin-1 (ANGPT1)	0.486	0.03	1	0.0009
Ficolin-3 (FCN3)	0.302	0.037	0.875	0.009
C-peptide	0.36	0.038	0.916	0.014
Complement C3 (C3)	0.269	0.048	0.777	0.059

OSAS Obstructive sleep apnea syndrome, *MCFSI* Mail-In Cognitive Function Screening Instrument, *AUC* Area under the curve

reported history of sleep apnea and sleep apnea treatment. Participants were selected for plasma collection by the presence ($N = 6$) or absence ($N = 6$) of an elevated MCFSI score $\geq$ 5. No patient had diabetes.

Proteomic analysis and statistical methods

Plasma was collected and stored at $-80\ °C$ until use. Proteomic analysis was done by Myriad RBM using targeted ELISA for 254 serum proteins. Correlations between various protein levels and presence of CI were analyzed using JMP software. A p value < 0.05 was considered to be statistically significant. Correlations were determined to be weak, moderate, or high when the r^2 coefficient was in the range of 0.04–0.15, 0.16–0.35, or 0.36–0.62, respectively. Area under the curve (AUC) was further used to assess correlations between different biomarkers and the presence of CI. AUC is defined as the probability of correctly ranking a randomly chosen diseased subject as higher or with greater suspicion than a randomly chosen non-diseased subject [14]. Pathway analysis of proteins was performed using the UniProt identifier and the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database for known and predicted *Homo sapiens* protein–protein interactions [15].

Results

Mean age of women who had OSAS and CI was 52.7 ± 3.56 years (mean ± SD) vs. 52.2 ± 2.14 years in those who had OSAS but no CI (p value 0.77). Mean MCFSI was 8.83 ± 2.42 in women with OSAS and CI as opposed to 2 ± 1.38 in those with OSAS and no CI (p value 0.0001). There was no difference in the prevalence of depression or obesity in the 2 groups of subjects. Average body mass index (BMI) in women with OSAS and CI was 36.6 ± 8.62 vs. 30.15 ± 6.09 in those with OSAS and no CI (p value 0.18). Hypertension was seen in 5 of the 6 subjects with OSAS and CI as opposed to in none of the subjects with OSAS and no CI (p value 0.003). Thus, the results of protein analysis were adjusted for hypertension as it was a significantly different covariate in the two groups. Diabetes, heart disease, and stroke were not reported by any of the subjects in this study.

Some proteins were found to decrease (Table 2) with increasing MCFSI scores (worse CI) while some were found to increase with increasing MCFSI scores (Table 3). Leucine rich alpha-2 glycoprotein was found to have a high correlation with CI with decreasing protein levels being seen with worse CI. Prostasin also had a high correlation with CI but with increasing protein levels being seen with worse CI. Other proteins with a moderate or weak correlation with CI are shown in Tables 2 and 3. Figure 1 depicts the heatmap for

the expression levels of various proteins in subjects with OSAS and with and without CI, compared to a midpoint value between the two groups.

Pathway analysis using the STRING database web interface revealed a central role for insulin, possibly a regulatory role (Fig. 2), with correlations seen between insulin (at the center of the network) and several other proteins.

Discussion

The field of proteomics is changing the perception of the pathogenesis of complex chronic health conditions. The advantage of an unbiased proteomic approach to CI in OSAS is that novel protein signatures, which provide a systems level overview, allow new insights into the pathophysiology of the disease. Our study indicates that CI in OSAS is associated with
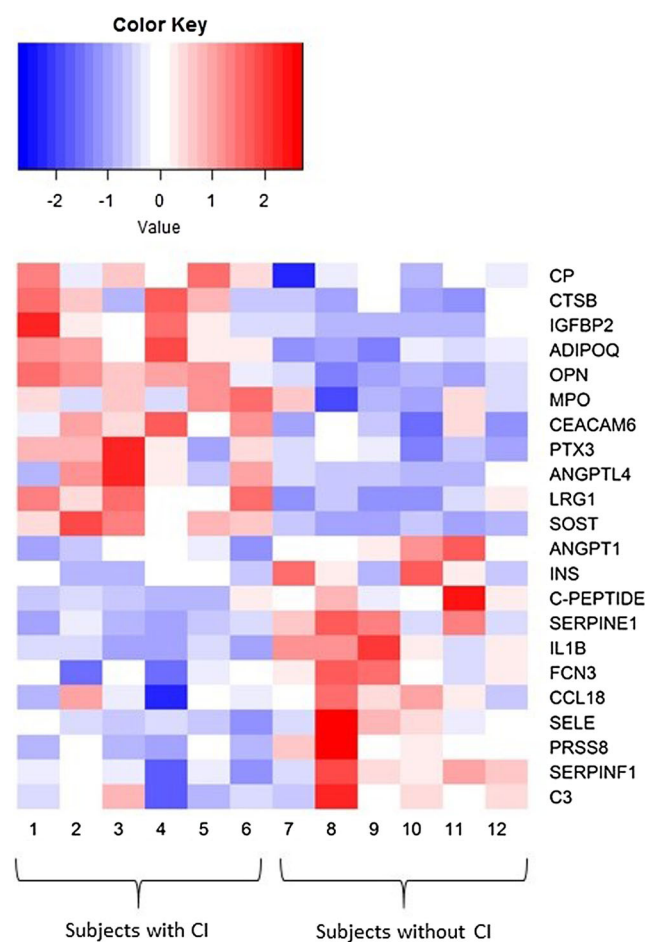
Fig. 1 Heatmap depicting the expression of upregulated and downregulated proteins with increasing MCFSI scores in OSAS subjects. Each column represents one subject with (Columns 1–6) or without (Columns 7–12) cognitive impairment (CI). Protein abbreviations are found in Tables 1 and 2. Red and blue boxes indicate relative under- and overexpression with respect to a reference which is calculated as the mid-point between the control and exposed groups
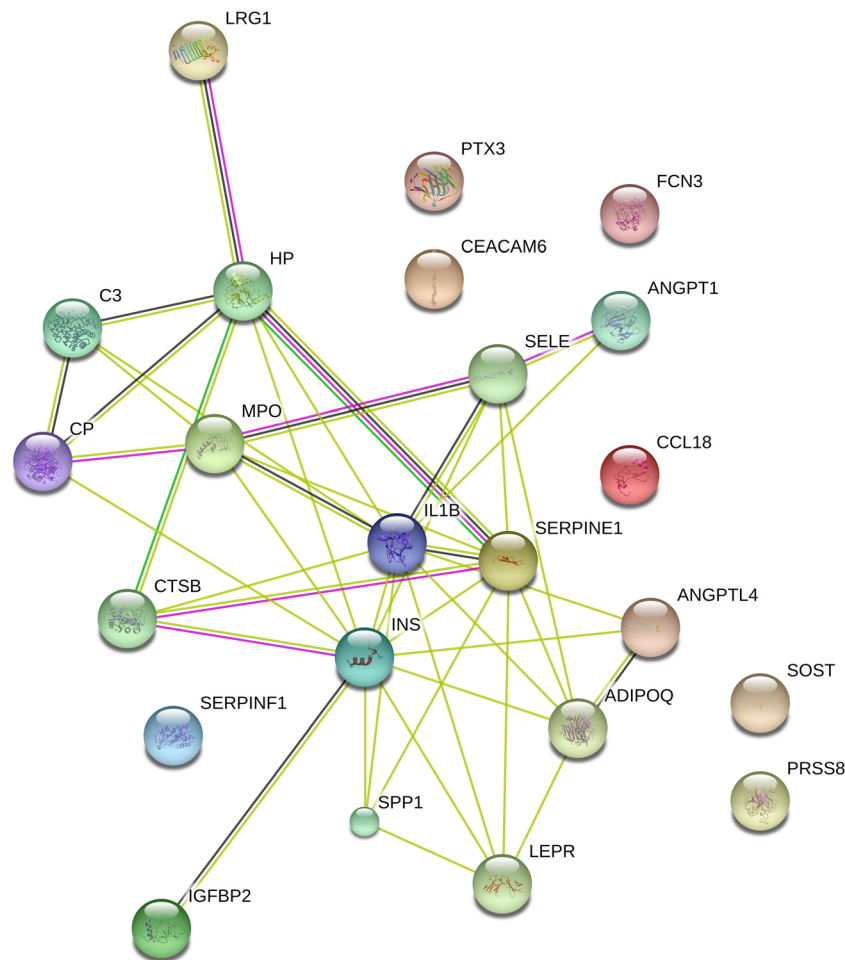
Fig. 2 Uniprot pathway analysis of top 11 upregulated and top 11 downregulated proteins in OSAS subjects with cognitive impairment show insulin having a central regulatory role, with small vessel ischemia, and obesity-driven inflammation playing important roles as well. Protein abbreviations are found in Tables 1 and 2. Some proteins which were found to play an intermediary role in the protein pathway but were not among the top underexpressed and overexpressed proteins were HP (Haptoglobin), SPP1 (Secreted phosphoprotein 1), and LEPR (Leptin receptor). Individual nodes represent proteins. Small nodes: proteins of unknown 3D structure; large nodes: proteins for which some 3D structure is known/predicted; colored nodes: query proteins and first shell of interactions; white nodes: second shell of interactions. Edges represent protein-protein associations. Known Interactions from curated databases, experimentally determined. Predicted Interactions gene neighborhood, gene fusions, gene co-occurrence. Others textmining, co-expression, protein homology

Insulin signaling pathways, despite the lack of diabetes in this patient cohort.

Insulin levels are associated with obesity, independent of diabetes. However, these two cohorts of post-menopausal women had no statistical difference in BMI. Insulin has biologic effects other than the regulation of glucose metabolism, including effects on cholesterol metabolism [16] and cognition [17]. In fact, brain insulin resistance has been shown to play a role in the pathogenesis of Alzheimer's disease (AD) [18]. Brain insulin resistance is not dependent on diabetes and may induce the formation of β amyloid peptide and tau phosphorylation in AD. Thus, studies have evaluated the use of nasal insulin in patients with mild cognitive dysfunction and AD and found improved cognition in these patients with intranasal administration of insulin [19]. Of course these results would need to be confirmed with larger clinical trials before insulin can be recommended as mainstream therapy for AD. Our study showed overexpression of insulin in patients with higher MCFSI scores which is consistent with findings from prior studies that have shown higher fasting insulin levels to be associated with a faster rate of cognitive decline [20].

Angiopoietin-1 was elevated in the CI group in this study. High angiopoietin-1 levels in patients with AD and an inverse correlation with the Mini-Mental State Examination scores have been reported in other studies [21]. Intermittent episodic hypoxia is the hallmark of OSAS. Upregulation of the hypoxia-inducible gene of angiopoietin-1 can result in hypoxia induced angiogenesis [22]. Thus, it is possible that the intermittent episodic hypoxia of OSAS is responsible for the overexpression of angiopoietin-1 seen in the CI group in this

study. Although higher levels of angiopoietin-1 are also seen in AD, vascular density is not higher in AD and instead is decreased [23]. Thus, complex vascular signaling mechanisms are likely involved in the occurrence of cognitive decline.

Angiopoietin-1 and some of the other protein signatures found in this analysis such as an elevated Plasminogen activator inhibitor 1 (PAI-1 or SERPINE1) level are also known to promote atherosclerosis [24, 25].

Interleukin-1 beta (IL1B), a pro-inflammatory cytokine, was also found to be overexpressed in subjects with CI in this study. This finding is consistent with studies of AD patients which have shown the inflammatory response to be upregulated with higher serum levels of several cytokines [26]. E-Selectin or endothelial-leukocyte adhesion molecule 1 (ELAM-1) recruits leukocytes to the site of injury in inflammation [27]. Overexpression of E-selectin and IL1B in this study suggests that further study of OSAS associations with the inflammatory response are warranted in the study of CI.

Certain serum proteins were found to be selectively underexpressed in subjects with CI in this study. Adiponectin is involved in glucose and fatty acid metabolism [28]. It is secreted from adipose tissue and levels of this protein have been found to be inversely correlated to BMI [29]. It protects against endothelial dysfunction and atherosclerosis [28] and in combination with leptin has been shown to reverse insulin resistance in mice [30]. Adiponectin levels were decreased with increasing CI in our study. In addition, the group with CI had trends toward a higher BMI than the group without CI.

Also underexpressed was cathepsin B (CTSB), a lysosomal cysteine protease [31] also known as amyloid precursor protein secretase that is involved in the proteolytic processing of amyloid precursor protein (APP) [32]. Incomplete proteolytic processing of APP may be causative in AD.

Thus, the CI in OSAS appears to be the result of multiple intricate pathways, involving insulin, endothelial dysfunction, atherosclerosis, inflammation, and proteolytic processes. A delineation of how exactly these processes interact with each other would require future large studies. However, pathway analysis is the mechanism by which proteomic signatures can sometimes be integrated. Although, Fig. 2 shows many divergent signals, the central themes of insulin playing a central regulatory role, small vessel ischemia, and obesity driven inflammation are prominent.

We will point out though that this analysis may be specific to postmenopausal women, which is an important observation as we begin to phenotype the many OSAS populations. Additional at-risk populations with OSAS should be studied, to see if other vulnerable populations with OSAS and CI share the same proteomic signatures.

The limitations of our study include the small sample size. In addition, OSAS risk was defined by Berlin questionnaire and CI was defined by MCFSI scores. Formal sleep studies and neuropsychological testing were not performed. Future

large studies should be undertaken to address these limitations and confirm our findings. Additionally, correlation analyses are weak surrogates for robust statistical differences between groups. Future cohorts of obese non-OSAS patients and non-obese OSAS patients will be needed to understand our findings in context.

Conclusion

In this pilot study, we have identified biomarkers for CI in OSAS that are shared by AD such as insulin, angiopoietin-1, and IL1B, that point to the possibility of a shared pathogenesis. If our results are confirmed by validation cohorts, then these biomarkers can assume a diagnostic role for early detection of CI in OSAS and can possibly be targeted for therapy. Future large studies are needed to delineate the exact interaction between these proteins and the molecular mechanisms by which CI occurs in OSAS.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest Dr. Lal has received grant support from Jazz pharmaceuticals and Invado pharmaceuticals and is a consultant for Jazz and Cipla pharmaceuticals. Drs. Strange, Hardiman, and Suchit Kumbhare have nothing to disclose.

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