Circulating anti-coatomer protein complex subunit epsilon (COPE) autoantibodies as a potential biomarker for cardiovascular and cerebrovascular events in patients with obstructive sleep apnea

（閉塞性睡眠時無呼吸症候群患者における心血管／脳血管イベントのバイオマーカーとしての抗COPE抗体の可能性）

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Abstract

Study Objectives: Although moderate to severe obstructive sleep apnea (OSA) is an independent risk factor for severe arteriosclerotic diseases such as cardiovascular disease (CVD) and stroke, the development of atherosclerosis-related diseases cannot yet be predicted in OSA patients. In a pilot study, we identified autoantibodies against the coatomer protein complex, subunit epsilon [circulating anti-coatomer protein complex subunit epsilon autoantibody (COPE-Ab)], a cytosolic complex that mediates protein transport in the Golgi compartment, as a potential novel biomarker of atherosclerosis. This study aimed to evaluate whether COPE-Ab levels had an association with cardiovascular and cerebrovascular events in OSA patients.

Methods: Eighty-two adult patients diagnosed with OSA via polysomnography and 64 healthy donors were studied. Serum COPE-Ab levels were measured using an amplified luminescence proximity homogeneous assay. Then, clinical factors related to atherosclerosis were evaluated with respect to COPE-Ab levels.

Results: Significant differences in COPE-Ab levels were observed in terms of OSA severity. COPE-Ab levels were significantly higher in OSA patients with CVD and/or stroke, hypertension, and a high body mass index. Univariate and multivariate logistic regression analyses of OSA patients identified elevated COPE-Ab level as a significant predictor of CVD and/or stroke.

Conclusions: An elevated COPE-Ab level may be a potential predictor of the risks of cardiovascular and cerebrovascular events in OSA patients. Therefore, patients with higher COPE-Ab levels may require more careful and intensive treatment.

Key words: sleep-related breathing disorders, atherosclerosis, cardiovascular disease, stroke, biomarkers

Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive obstruction of the upper airway during sleep, which causes recurrent intermittent hypoxia. OSA patients often exhibit excessive daytime sleepiness related to sleep fragmentation because of frequent arousal with apnea. Although OSA is considered a local and upper airway disorder, moderate to severe OSA has recently been recognized as a systemic disease that is associated with the potential risks of brain stroke, fatal or non-fatal cardiovascular events, and death from causes mainly related to the atherosclerotic process.[1-4]

OSA-related intermittent hypoxia promotes oxidative stress by promoting the production of reactive oxygen species and angiogenesis, inducing sympathetic activation with blood pressure elevation. It causes systemic, adipose tissue, and vascular inflammation with endothelial dysfunction; taken together, these factors lead to the development of atherosclerosis and eventually cardiovascular and cerebrovascular diseases.[5-7] Although various modalities for the evaluation of arterial atherosclerosis, such as brachial-ankle pulse wave velocity (baPWV), the ankle-brachial pressure index (ABI), carotid artery ultrasonography, fundus examination, and microalbuminuria status, have been introduced, their use cannot sufficiently predict the development of devastating conditions such as cardiovascular disease (CVD) and stroke. Additionally, considerable time and effort may be required to obtain reproducible and reliable results using these modalities. In the meantime, several studies have demonstrated the clinical benefit of continuous positive airway pressure (CPAP) therapy for OSA patients in relation to atherosclerosis-related parameters such as inflammatory markers, lipid profile, and blood pressure.[8-10] However, reported CPAP therapy usage frequency ranges from 29–85%, suggesting that positive adherence has not yet been achieved.[11] Therefore, the development of a simple predictive modality for OSA patients who require continuous CPAP therapy is needed.

Circulating (i.e., serum) autoantibodies against atherosclerosis-specific antigens have been considered candidate markers of cardiovascular risk. Previously, we have identified autoantibodies recognized by IgG antibodies in the sera of patients with atherosclerosis-related diseases such as myocardial infarction, stroke, and diabetes.[12, 13] In our previous studies, we were the first to introduce the serological identification of antigens by recombinant cDNA expression cloning (SEREX) method to screen for clones with immunoreactivity against IgG antibodies in patient sera. Subsequently, we used an amplified luminescence proximity homogeneous assay (AlphaLISA) to evaluate the levels of selected antibodies in these patients. In another pilot study, we used SEREX screening to identify the coatomer protein complex subunit epsilon (COPE; accession number CR456886) (clone S33b7) as an antigen recognized by serum antibodies (Abs) in patients with atherosclerosis.

In this report, we describe our investigation of circulating COPE-Ab levels in the sera of general OSA patients in an attempt to evaluate whether COPE-Ab levels had an association with cardiovascular and cerebrovascular events in OSA patients.

Methods

Ethical approval

All study procedures involving human participants were conducted in accordance with the ethical standards of institutional (The Local Ethical Review Board of Chiba University, Graduate School of Medicine) and/or national research committees and conformed to the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients and healthy donors

Eighty-two Japanese adult patients diagnosed with OSA by polysomnography (PSG) in our

Hospital from June 2012 to January 2014 were studied (56 men and 26 women; median age: 59.0 years). Sixty-four healthy volunteer donors (HDs) who underwent medical checkups and had no history of OSA were used as control subjects (38 men and 26 women; median age: 42.5 years). All HDs were enrolled at Chiba University. Patients with autoimmune diseases were excluded from this study, and all individual study participants provided informed consent.

Blood sampling and purification

Blood samples were collected from each patient upon study admission. Each serum sample was centrifuged at 3000 × g for 10 min at room temperature, and the supernatant was stored at -80˚C until use. Repeated thawing and freezing of samples was avoided.

Clinical data

Classical risk factors for atherosclerosis, including age, sex, body mass index (BMI), smoking status, hypertension, diabetes, hyperlipidemia, CVD, and stroke, were determined from clinical records. Patients were divided into three groups according to smoking status (i.e., never-smoked, ex-smoker, and current-smoker). Hypertension was defined as a history of systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or antihypertensive agent use, and diabetes was defined as the use of antidiabetic therapy or a history of diabetes. Hyperlipidemia was defined as a history of a total cholesterol level > 220 mg/dL, triglyceride level > 150 mg/dL, or lipid lowering agent use. CVD was defined as a history of myocardial infarction or angina pectoris. Stroke was defined as a history of cerebral infarction or cerebral hemorrhage. Cardiovascular and cerebrovascular events were defined as having a past history of CVD and/or stroke.

PSG was scored according to the 2007 American Academy of Sleep Medicine (AASM) alternative criteria.[14] Apnea was defined as a reduction in the nasal airflow to < 10% of the baseline for ten

seconds or more, while hypopnea was defined as a reduction in the nasal airflow signal amplitude of ≥ 50% for ten seconds or more in association with either a ≥ 3% oxygen desaturation or electroencephalographic (EEG) arousal. OSA was defined as an apnea-hypopnea index (AHI) five events or more per hour combined with predominantly obstructive respiratory events. OSA severity was classified according to AHI values as follows: mild, 5–15; moderate, > 15–30; and severe, > 30.

Expression and purification of antigenic glutathione-S-transferase (GST)-fusion proteins

Recombinant GST-tagged proteins were constructed by recombining pBluescript insertion sequences into the pGEX-4T3 plasmid (GE Healthcare Life Sciences, Pittsburgh, PA, USA). A region comprising bases 35–1131 of COPE mRNA was cloned into pBluescript II. As the actual coding sequence was located between bases 43 and 969 of the total 1134 bases, the full-length coding sequence of COPE was recombined into pGEX-4T3. The gene product was purified as described in our previous reports.[12, 15, 16]

Amplified luminescence proximity homogeneous assay (AlphaLISA)

AlphaLISA was performed in 384-well microtiter plates (white opaque OptiPlate™, PerkinElmer, Waltham, MA, USA) containing 2.5 µL of 1/100-diluted sera and 2.5 µL of GST or a GST-fusion protein (10 µg/mL) in AlphaLISA buffer (25 mM HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/mL dextran-500, and 0.05% Proclin-300). The resulting reaction mixture was incubated at room temperature for six-eight hours. Next, anti-human IgG-conjugated acceptor beads (2.5 µL of 40 µg/mL) and glutathione-conjugated donor beads (2.5 µL of 40 µg/mL) were added, and the samples were subjected to an additional 14-day incubation at room temperature in the dark. The chemical emissions of samples were read on an EnSpire Alpha microplate reader (PerkinElmer) as previously described.[13, 15, 16] Specific reactions were calculated by subtracting the Alpha values of GST control samples from those of samples containing GST-fusion proteins.

Statistical analyses

All statistical analyses were performed using the JMP Pro 12.2.0 software program (SAS Institute Inc., Cary, NC, USA), and all data are expressed as medians (interquartile ranges). The Mann–Whitney U or Kruskal–Wallis test was used to determine the significance of differences in baseline characteristics between groups. The association between COPE-Ab levels and OSA severity was compared among HDs, the mild OSA group, moderate OSA group, and severe OSA group using Kruskal–Wallis test. Each OSA group was compared with HDs as a post hoc analysis using Steel's test. The pooled all OSA group was compared with HDs using Mann–Whitney U test. Correlations were evaluated using a Spearman's correlation analysis, and Fisher's exact test was used to determine differences in the proportions of groups. The cut-off value of COPE-Ab levels for predicting CVD and/or stroke among all OSA patients was determined using receiver operating characteristic (ROC) curve analysis at the value that maximized the sums of sensitivity and specificity. Univariate and multivariate logistic regression analyses were used to identify the set of variables that would classify patients according to CVD and/or stroke status. Eight covariates were included in the models: age (year), sex, obesity (BMI $\geq$ 25 kg/m^2), smoking (current or ex-smoker), hypertension, diabetes, hyperlipidemia, and elevated COPE-Ab levels. All tests were two-tailed, and statistical significance was defined as a p value of <0.05.

Results

Characteristics of OSA patients and HDs

Patients with OSA were divided into three groups corresponding to mild, moderate, and severe OSA; clinical characteristics of OSA patients and HDs are shown in Table 1. Sixty-four HDs and 82 OSA patients (11 mild, 17 moderate, and 54 severe OSA patients) were enrolled in this study. OSA

patients were significantly older and had higher BMI index than those of HDs. The histories of each atherosclerosis-related disease were more frequently observed as OSA severity increased. An AlphaLISA analysis of the serum COPE-Ab levels revealed significantly higher levels in moderate OSA, severe OSA, and the pooled all OSA patients relative to HDs ($p = 0.030$, $p < 0.001$, and $p < 0.001$, respectively) (Fig. 1). Regarding some outliers of COPE-Ab levels in HDs and OSA patients in Fig. 1, a sensitivity analysis with log transformation of the COPE-Ab data was performed. After the log transformation, there were less serious outliers, and the data were close to a normal distribution. The results did not change much: significant differences were observed among the four groups using ANOVA ($p < 0.001$), and post hoc analysis using Dunnett's test revealed significant differences between the severe OSA and HD groups ($p < 0.001$) and moderate OSA and HD groups ($p = 0.031$). Student's t-test revealed significant differences between the pooled all OSA and HD groups ($p < 0.001$).

Association of COPE-Ab levels and clinical parameters in OSA patients

The relationships of COPE-Ab levels of OSA patients with clinical parameters other than disease severity in OSA patients are shown in Fig. 2. A moderate association was observed between COPE-Ab level and BMI ($\rho = 0.33$, $p = 0.003$, Fig. 2c), mean SpO_2 ($\rho = -0.36$, $p < 0.001$, Fig. 2d), lowest SpO_2 ($\rho = -0.31$, $p = 0.004$, Fig. 2e), and arousal index ($\rho = 0.29$, $p = 0.008$, Fig. 2f), whereas significantly higher COPE-Ab levels were observed in patients with hypertension ($p = 0.013$, Fig. 2h), CVD ($p = 0.039$, Fig. 2k), and CVD and/or stroke ($p = 0.046$, Fig. 2l). In contrast, no significant differences in COPE-Ab levels were observed with respect to other parameters, including age, sex, smoking status, diabetes, or hyperlipidemia.

Associations between CVD and/or stroke and clinical parameters in OSA patients

The cut-off value of COPE-Ab levels for predicting CVD and/or stroke was determined to be 1050

using ROC curve analysis. The area under the curve (AUC) was 0.676 (95% confidence interval [CI]: 0.468–0.832).

The results of the univariate and multivariate logistic regression analyses are shown in Table 2. Using the COPE-Abs cut-off value derived from the ROC curve analysis, this univariate logistic regression analysis revealed associations between elevated COPE-Ab levels and male sex with the risk of CVD and/or stroke (odds ratio [OR]: 4.50, 95% CI: 1.32–18.1, p = 0.016 and OR: 6.82, 95% CI: 1.23–127.8, p = 0.025, respectively). The multivariate logistic regression analysis, which included variables that differed significantly in the univariate analysis, revealed an independent association between elevated COPE-Ab levels and CVD and/or stroke (OR: 3.90, 95% CI: 1.11–16.0, p = 0.034).

Discussion

In this study, we discovered two important features of COPE-Abs in patients with untreated OSA. First, COPE-Ab levels were significantly higher in OSA patients, particularly those with moderate to severe OSA, than in HDs. Furthermore, OSA patients with CVD and/or stroke, hypertension, and a high BMI had elevated levels of COPE-Ab. Second, an elevated COPE-Ab level was the significant predictor of a history of CVD and/or stroke in a multivariate analysis of OSA patients. According to our results, COPE-Abs might therefore be a biomarker of the risk of cardiovascular and cerebrovascular events in OSA patients.

According to recent studies, circulating autoantibodies exist in the sera of patients with atherosclerosis, including autoantibodies against phospholipids in patients with acute coronary syndrome,[17, 18] apolipoprotein A-1 in patients exhibiting atherosclerotic plaque vulnerability,[19] and

oxidized low-density lipoprotein, which is associated with plaque formation and coronary risk in some patients with systemic lupus erythematosus.[20] Previously, we have also reported the identification of atherosclerosis antigens via expression cloning from a phage cDNA library, as well as the association of the levels of antibodies against several autoantigens (e.g., RPA2, ATP2B4, BMP-1) with atherosclerosis-related risk factors, including stroke, hypertension and/or smoking habits.[12,16] Antibody markers appear to be highly sensitive and therefore are expected to be predictive markers for other atherosclerotic diseases, such as the onset of stroke and CVD, in patients with OSA. In the preliminary stage of the present study, COPE-Ab levels were the most closely associated with OSA severity among our candidate markers. Therefore, we believed that this antibody marker might be useful for evaluating lethal atherosclerotic disease onset in patients with OSA, and thus, we initiated the present study and analysis.

COPE (36 kDa) is one of seven subunits of the coatomer protein complex known as coat protein complex I (COPI). COPI, which is among the best-characterized coat complexes, is a cytosolic complex that coats Golgi-derived vesicles and is involved in protein transport from the Golgi apparatus to the endoplasmic reticulum via recruitment by Arf1.[21-23] Although the precise cellular mechanisms of COPI, and circulating COPE-Ab, in OSA patients were not revealed by this present study, lipid inflammation might be a key element associated with elevated COPE-Ab levels in this patient population. Previous studies have reported that the Arf1–COPI vesicular transport machinery regulates droplet morphology and lipid storage/utilization within the vesicle-trafficking pathway.[24-26] This implies a link between COPI and lipid storage (e.g., atherosclerosis-related) diseases. In the mechanism underlying atherosclerosis, macrophages and smooth muscle cells infiltrate into vascular endothelial cells to uptake lipids, resulting in the differentiation into foam cells. Foam cells aggregate to form an atheroma, leading to the occlusion of blood vessels.[27] OSA is often accompanied by obesity; therefore, serum levels of COPI and its subunit, COPE, may be elevated to store excessive

lipids in the cells in OSA patients. This speculation is consistent with the association of elevated COPE-Ab levels and BMI in the present study (Fig 2c). Furthermore, preceding a CVD and/or stroke, atheromatous plaques are partially ruptured, which can lead to the leakage of excessively expressed COPE into extracellular or intravascular spaces. Then, repetition of this COPE leakage may result in an elevated expression of COPE-Ab in serum.

Additionally, chronic intermittent hypoxia, which is experienced by most OSA patients, has been known to modulate lipid metabolism via lipid peroxidation at a cellular level[28]; additionally, this condition increases sympathetic activity, which affects the blood pressure and heart rate, promotes free radical production and adhesion molecule expression, and induces insulin and leptin resistance, by altering peripheral (carotid bodies) and central (nucleus tractus solitarius, hypothalamus, and ventral medulla) chemoreflex pathways.[29, 30] Another study found that intermittent hypoxia caused adipose tissue inflammation in OSA patients by increasing macrophage recruitment and the secretion of interleukin-6 and tumor necrosis factor-α, leading to the development of atherosclerosis.[31] These potential COPE-mediated effects of chronic intermittent hypoxia on lipid metabolism might be reflected in the observed higher levels of COPE-Ab in untreated OSA patients, although hyperlipidemia *per se* did not significantly correlate with the COPE-Ab level in the present study ($p = 0.193$, Fig. 2j). In Fig. 1, the COPE-Ab levels in mild OSA patients did not differ from those in HDs. This results can be explained by the level of (intermittent and/or sustained) hypoxia, because elevated COPE-Abs levels were associated with lower oxygen levels during sleep, as shown in Fig. 2d and e, which are relatively milder in mild OSA patients than those in moderate to severe OSA patients. Additionally, this result of mild OSA is consistent with a previous study; mild OSA has no clear association with the risk of atherosclerosis-related diseases.[2]

As the prevention of future cardiovascular and cerebrovascular events is the main purpose of OSA

treatment, COPE-Ab might serve as a simple and easy biomarker for the evaluation of OSA patients who clearly require continuous CPAP therapy. Generally, atherosclerosis in OSA patients is evaluated via the clinical analyses of coronary artery calcium levels, carotid intima media thickness (IMT), baPWV, and flow-mediated dilation.[32] Of these, increases in baPWV, IMT, and carotid diameter were reported to be potential early signs of atherosclerosis in OSA patients.[33] However, when these parameters are evaluated, the autoantibody approach described in this study is clearly more simple and convenient, as it only requires a patient serum sample. Several markers of inflammation and endothelial dysfunction related to cardiovascular or cerebrovascular disease have also been described as atherogenic pro-inflammatory markers in patients with OSA; these include soluble tumor necrosis factor receptor, tumor necrosis factor-beta, interleukin-6, and soluble intercellular cell adhesion molecule-1. However, the usefulness of these biomarkers as simple predictors of future cardiovascular and cerebrovascular events remains to be determined. Accordingly, analysis of circulating COPE-Ab levels could prove useful for predicting the risk of lethal events in OSA patients, although prospective cohort studies are needed to confirm the efficacy of COPE-Ab as a marker of atherosclerosis in this population.

We must note some limitations associated with our study. First, the number of patients with mild or moderate OSA was relatively small, as was the number of patients with stroke (just five of 82 OSA patients). Accordingly, the cut-off value of COPE-Ab levels for predicting CVD and/or stroke among all OSA patients determined via ROC curve analysis could be inaccurate because of the small number of CVD and/or stroke events. In addition, as 64 controls were HDs, potential confounding factors between OSA patients and controls (e.g., age, BMI, hypertension, diabetes, and hyperlipidemia) were not adjusted in the analysis in the present study. Second, some classical risk factors (e.g., hyperlipidemia and diabetes mellitus) of atherosclerosis were not associated with elevated COPE-Ab levels in this study. As antihypertensive agents, statins, and antiplatelet agents are

generally known to affect the pathogenesis of atherosclerosis,[34-37] we must consider the potential modulatory effects of these drugs on COPE-Ab levels. Third, we did not conduct physiological testing, such as ABI, baPWV, or coronary artery calcification, to evaluate atherosclerosis in subjects subjected to the COPE-Abs analysis. Nevertheless, these tests might be expected to confirm the results of the present study. Finally, the study population included only Japanese patients. Further studies are required in patients who are not taking drugs that can affect atherosclerosis and in other ethnic groups.

In conclusion, patients with OSA had higher circulating COPE-Ab levels than did HDs. Notably, the COPE-Ab levels were significantly higher in OSA patients with CVD and/or stroke, hypertension, and a high BMI. The results suggest that elevated COPE-Ab levels could serve as a potential biomarker of the risk of cardiovascular and cerebrovascular events in this patient population. Therefore, patients with higher COPE-Ab levels may require more careful and intensive treatment.

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Figure legends

Fig. 1 Association between COPE-Ab levels and OSA severity. OSA patients were classified into three groups according to OSA severity; these groups were then compared with HDs. Significant differences were observed among the four groups using Kruskal–Wallis test ($p < 0.001$). The post hoc analysis using Steel's test revealed significant differences between the moderate and severe OSA versus HD groups. Mann–Whitney U test revealed that the pooled all OSA group was also significantly higher than HDs. Horizontal lines represent the median, boxes represent the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and dots represent the outliers. COPE-Abs: autoantibodies against coatomer protein complex, subunit epsilon; OSA: obstructive sleep apnea; HD: healthy volunteer donors.

Fig. 2 Associations between COPE-Ab levels and clinical parameters other than OSA severity in OSA patients. Correlations were evaluated between COPE-Abs and age (a), sex (b), BMI (c), mean SpO_2 (d), lowest SpO_2 (e), arousal index (f), smoking status (g), hypertension (h), diabetes (i), hyperlipidemia (j), CVD (k), and CVD and/or stroke (l). A moderate association was observed between COPE-Ab levels and BMI, mean SpO_2, lowest SpO_2, and arousal index. In addition, significantly higher COPE-Ab levels were observed in patients with hypertension, CVD, and CVD and/or stroke. Mann–Whitney U test (b, h–l), Kruskal–Wallis test (g), and Spearman's correlation analysis (a, c–f) were used. Horizontal lines represent the median, boxes represent the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and dots represent the outliers. COPE-Abs: autoantibodies against coatomer protein complex, subunit epsilon; OSA: obstructive sleep apnea; BMI: body mass index; CVD: cardiovascular disease.

Table 1 Baseline characteristics of subjects

	HD (n = 64)	All OSA (n = 82)	Mild OSA (n = 11)	Moderate OSA (n = 17)	Severe OSA (n = 54)
Age (years)	42.5 (35.3–55.8)	59.0 (49.8–66.5)[c]	59.0 (58.0–64.0)[b]	59.0 (47.5–65.0)[b]	59.5 (49.0–69.0)[c]
Male sex	38 (59.4%)	56 (68.3%)	4 (36.3%)	9 (52.9%)	43 (79.6%)[a]
BMI (kg/m^2)	23.1 (20.6–25.5)	25.9 (23.9–29.4)[c]	22.8 (21.7–25.2)	26.0 (22.7–29.3)[a]	26.3 (25.0–31.7)[c]
Polygraphic data					
AHI (events/h)		36.7 (22.6–50.4)	10.0 (8.2–12.6)	22.4 (17.5–24.6)	46.2 (36.9–60.2)
Mean SpO_2 (%)		94.0 (93.0–96.0)	96.0 (95.0–97.0)	95.0 (94.5–96.0)	94.0 (92.0–95.0)
Lowest SpO_2 (%)		78.0 (69.0–83.0)	88.0 (85.0–91.0)	80.0 (78.0–83.0)	74.0 (66.3–79.3)
Arousal index (events/h)		37.3 (22.2–50.3)	15.2 (11.3–22.3)	21.6 (16.6–31.6)	46.7 (33.8–61.2)
Smoking					
Never-smoked	42 (70.0%)	42 (51.2%)	7 (63.6%)	12 (70.6%)	23 (42.6%)
Ex-smoker	10 (16.7%)	34 (41.5%)	3 (27.3%)	3 (17.6%)	28 (51.9%)
Current-smoker	8 (13.3%) (n = 60)	6 (7.3%)[b]	1 (9.1%)	2 (11.8%)	3 (5.6%)[b]
Hypertension	8 (12.5%)	30 (36.6%)[b]	4 (36.3%)	4 (23.5%)	22 (40.7%)[b]
Diabetes	1 (1.6%)	17 (20.7%)[b]	0 (0.0%)	1 (5.9%)	16 (29.6%)[c]
Hyperlipidemia	2 (3.1%)	22 (26.8%)[a]	3 (27.3%)[a]	6 (35.3%)[b]	13 (24.1%)[b]
CVD	0 (0.0%)	10 (12.2%)[b]	1 (9.1%)	0 (0.0%)	9 (16.7%)[b]
Stroke	0 (0.0%)	5 (6.1%)	0 (0.0%)	0 (0.0%)	5 (9.3%)[a]

Data represent the median (interquartile range) for numerical data and n (%) for categorical data, [a]$p < 0.05$ versus HD, [b]$p < 0.01$ versus HD, [c]$p < 0.001$ versus HD. HD: healthy volunteer donor, OSA: obstructive sleep apnea, BMI: body mass index, AHI: apnea-hypopnea index, CVD: cardiovascular disease.

Table 2 Logistic regression analysis of CVD and/or stroke predictions in patients with OSA

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	p value	OR	95% CI	p value
Age (per year)	1.01	0.96–1.07	0.66			
Male sex	6.82	1.23–127.8	0.025	5.69	0.98–108.2	0.053
Obesity (BMI ≥25, kg/m^2)	0.85	0.26–3.09	0.80			
Smoking (Current or Ex-smoker)	2.76	0.81–11.0	0.10			
Hypertension	1.10	0.30–3.67	0.88			
Diabetes	2.97	0.78–10.6	0.11			
Hyperlipidemia	1.91	0.52–6.55	0.32			
Elevated COPE-Ab levels (≥ 1050)[a]	4.50	1.32–18.1	0.016	3.90	1.11–16.0	0.034

[a]The COPE-Ab level cut-off value was set at 1050, based on ROC curve analysis. CVD: cardiovascular disease, OSA: obstructive sleep apnea, BMI: body mass index, OR: odds ratio, CI: confidence interval.

Fig.1

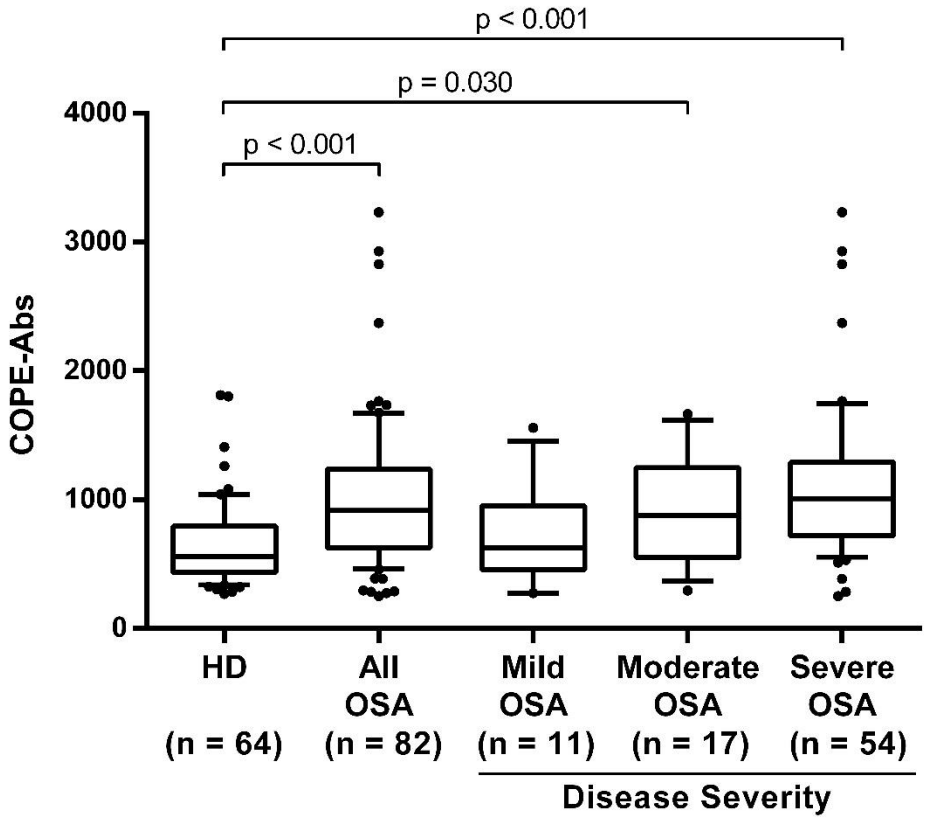
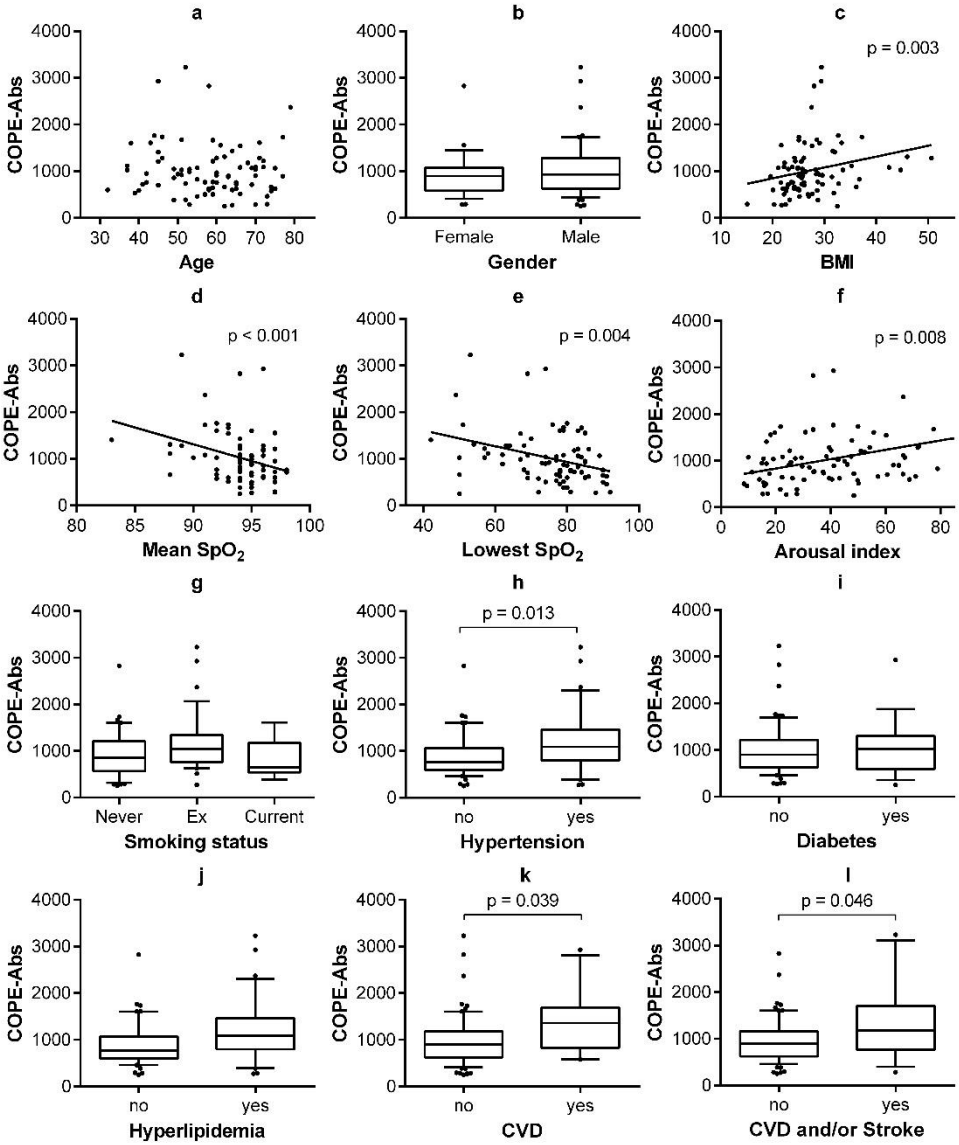

p < 0.001
p = 0.030
p < 0.001
4000
3000
2000
1000
0
COPE-Abs
HD
(n = 64)
All
OSA
(n = 82)
Mild
OSA
(n = 11)
Moderate
OSA
(n = 17)
Severe
OSA
(n = 54)
Disease Severity

Fig.2



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