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# TNFRSF11B: A potential plasma biomarker for diagnosis of obstructive sleep apnea

Wan-Wan Wen[a], Yu Ning[a], Qing Zhang[b], Yun-Xiao Yang[a], Yi-Fan Jia[a], Hai-Li Sun[c], Yan-Wen Qin[d], Fang Fang[c], Ming Zhang[a,*], Yong-Xiang Wei[c,**]

[a] Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China
[b] Department of Neurosurgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[c] Department of Otolaryngology Head & Neck Surgery, Key Laboratory of Upper Airway Dysfunction-related Cardiovascular Diseases, Beijing Institute of Heart Lung and Blood Vessel Disease, Beijing Anzhen Hospital, Capital Medical University, Beijing, China
[d] Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, Beijing, China

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ABSTRACT

*Background:* Obstructive sleep apnea (OSA) was characterized by chronic intermittent hypoxia, which was an independent risk factor for endothelial dysfunction. Circulating TNFRSF11B might play an important role in promoting endothelial cells dysfunction. We explored the role of plasma TNFRSF11B as a potential mechanism of endothelial dysfunction in OSA patients.

*Methods:* The study population consisted of 120 patients with varying severity of OSA and 40 control subjects. Plasma TNFRSF11B levels were measured using human Magnetic Luminex assay.

*Results:* Our data showed that plasma TNFRSF11B levels were significantly higher in patients with OSA. After adjusting confounding factors, plasma TNFRSF11B levels were independently associated with the presence of OSA (Beta:0.434, 95% CI: 664.096 to 1076.247; $P < 0.001$) and plasma TNFRSF11B levels were positively associated with the apnea-hypopnea index (Beta:0.486, 95% CI: 0.007 to 0.017; $P < 0.001$). Furthermore, plasma TNFRSF11B showed higher discriminatory accuracy in predicting the presence of OSA (AUC:0.964).

*Conclusions:* Plasma TNFRSF11B levels were significantly associated with the presence of OSA and its severity. TNFRSF11B could be a plasma biomarker with a positive diagnostic value for premature vascular endothelial dysfunction in patients with OSA.

## 1. Introduction

Obstructive sleep apnea (OSA) was characterized by repetitive collapse of the upper airway during sleep leading to snoring, chronic intermittent hypoxia (CIH), oxidative stress, increased systemic inflammation and endothelial dysfunction [1,2]. These pathological changes were potential pathogenesis for increased risks of hypertension, diabetes mellitus and cardiovascular disorders [3,4]. Endocan, an endothelial-derived protein, which was potential endothelial cells dysfunction marker [5,6]. Recent studies showed that Endocan was an independent and significant indicator of OSA and its severity [7].

Endocan was a soluble proteoglycan, secreted by human vascular endothelial cells. [6] Clinical studies have reported that Endocan levels were significantly higher in patients with OSA, but it significantly decreased after 3 months of continuous positive airway pressure treatment [8]. Our previous study showed that Endocan was significantly upregulated under intermittent hypoxia in endothelial cells and Endocan might be a potential target for intermittent hypoxia-induced endothelial dysfunction [9].

Tumor necrosis factor receptor superfamily member 11b

*Abbreviations:* OSA, Obstructive sleep apnea; CIH, Chronic intermittent hypoxia; TNFRSF11B, Tumor necrosis factor receptor superfamily member 11b; PSG, Polysomnography; BMI, Body mass index; ESS, Epworth sleepiness scale; AHI, Apnea-hypopnea index; hsCRP, high-sensitivity C-reactive; ROC, Receiving operator curves; AUC, Area under curve; CHD, Coronary heart disease; CI, Confidence interval

* Correspondence to: Ming Zhang, Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, No.2, Anzhen Road, Chaoyang District, 100029, Beijing, China.

** Correspondence to: Yong-Xiang Wei, Department of Otolaryngology Head & Neck Surgery, Key Laboratory of Upper Airway Dysfunction-related Cardiovascular Diseases, Beijing Institute of Heart Lung and Blood Vessel Disease, Beijing Anzhen Hospital, Capital Medical University, No.2, Anzhen Road, Chaoyang District, 100029, Beijing, China.

*E-mail addresses:* zhangming2279@hotmail.com (M. Zhang), weiyongxiang@vip.sina.com (Y.-X. Wei).

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(TNFRSF11B) was a glycoprotein that exerts pleiotropic effects on bone metabolism, endothelial cell dysfunction, vascular inflammation and atherosclerosis [10–12]. Previous studies suggested that a pathological increase of serum TNFRSF11B levels might play an important role in promoting leukocyte/endothelial cell adhesion and endothelial cells dysfunction [13,14]. Thus, both circulating Endocan and TNFRSF11B may be the potential markers of endothelial cells dysfunction.

This study aimed to investigate whether the plasma TNFRSF11B levels were associated with the presence and severity of OSA and whether plasma TNFRSF11B could predict endothelial dysfunction in OSA patients.

## 2. Methods

### 2.1. Study design and participants

We conducted an observational cross-sectional study in adults with OSA. Between March 2017 and November 2017, 240 participants from Beijing Anzhen hospital underwent a polysomnography (PSG) due to suspicion OSA were included in the current analysis. Patients with age of < 18y and those with malignant tumors, chronic obstructive pulmonary disease, bronchial asthma and interstitial lung disease were excluded [15,16]. Finally, 160 patients were enrolled in this study. Baseline demographics, clinical history and risk factor information were extracted from self-administered questionnaires. Body weight (kg), height (cm), body mass index (BMI), blood pressure and left ventricular ejection fraction were measured or calculated using standardized and reproducible study protocols. Serum lipid, high-sensitivity C-reactive (hsCRP) protein and other routine biochemical parameters were measured in a biochemical analyzer (Hitachi-7600, Tokyo, Japan) using blinded quality control specimens in the Department of the Biochemical Laboratory at Beijing Anzhen Hospital. The project was approved by the Chinese Clinical Trial Registry (No. ChiCTRROC-17,011,027). The study protocol was approved by the Medicine Ethics Committee of Beijing Anzhen Hospital and adhered to the Declaration of Helsinki. All participants provided written informed consent.

### 2.2. Sleep data

All participants included in the study underwent detailed sleep evaluated. Neck circumference (cm) was measured between the mid-cervical spine and mid-anterior neck, in the standing upright position [17]. The Epworth Sleepiness Scale (ESS)were recorded for each study participant. ESS score > 10 indicated excessive daytime sleepiness [18]. All participant underwent full-night attended PSG [SOMNO screen plus; SOMNO medics] in the Sleep Laboratory [19,20]. Alcohol or sleeping medicines were prohibited during PSG performance. Respiratory movements or respiratory effort airflow, electroencephalogram, electrocardiogram, and oxygen saturation were continuously measured and recorded by a computerized polysomnogram. Sleep stages and cardiopulmonary events were manually evaluated by certified technicians according to the 2012 American Academy of Sleep Medicine criteria [21]. Hypopnea was defined as ≥3% oxygen desaturation sustaining for ≥10 s. Apnea was defined as a complete cessation of airflow or airflow decrease ≥ 90% relative to the baseline amplitude and persisting for ≥10 s. The number of hypopneas plus apneas per hour of sleep was defined as the apnea-hypopnea index (AHI). Participants with an AHI ≥ 5 events/h were diagnosed as having OSA. OSA was divided into mild OSA(5 ≤ AHI < 15 events/h), moderate OSA(15 ≤ AHI < 30 events/h), and severe OSA(AHI ≥30 events/h).

### 2.3. Collection and storage of plasma sample

Fasting whole blood samples were collected by venipuncture and anticoagulated using ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged at 3000*g* for 10 min. 500uL of the plasma was immediately transferred into a clean polypropylene tube and stored at −80 °C until use. Samples were thawed at 4 °C prior to assay performance.

### 2.4. Luminex assays

Magnetic Luminex® Assays was a magnetic bead-based antibody microarray founded upon the sandwich immunoassay principle, which can be used to assess the levels of biomarkers in a single sample [22,23]. The plasma levels of TNFRSF11B and Endocan have previously been evaluated in unique cytokines panels using Luminex techniques [24–26]. In our study, we custom-made a unique blend of magnetic bead cytokines panel including TNFRSF11B and Endocan to screen biomarkers that can be used to predict OSA (R&D Systems, Inc. Minneapolis, MN, USA) (Additional file 1: Table S1). In order to ensure the accuracy and validity of the results, we evaluated the Luminex multiplex assay system by the standard curve and intra-assay variability. Firstly, the standard curve was critical for quantitation measurements. The assay sensitivity and detection range of TNFRSF11B and Endocan were evaluated and the results were shown in Additional file 1: Table S2. Reading out of range of the standard curve were excluded from all subsequent analyses. Secondly, in order to determine the precision of the standards and cytokines levels values obtained by the Luminex platforms, we calculated intra-assay performance using coefficient of variation (CV%) to determine the precision of results. Intra-assay CV < 10% being acceptable [27]. In our study, the intra-assay CV of standard was < 4.0%. The intra-assay CV of TNFRSF11B and Endocan were shown in Additional file 1: Fig. S1. In addition, all plasma samples were optimally diluted to ensure cytokines levels fell within the detection dynamic range of the assay. All standards and samples were run in duplicate according to the manufacturer's instructions [https://resources.rndsystems.com/pdfs/datasheets/lxsahm.pdf]. Briefly, microparticles cocktail, diluted samples and cytokines standards were pipetted into a 96-wells flat-bottom plate, and then incubated for 2 h, the plates were washed and the diluted biotin antibody cocktail was added. After incubated for an hour, plates were washed and streptavidin-Phycoerythrin was added to incubate for 30 min, then washed using the wash buffer. Resuspend the microparticles by adding 100 μL of wash buffer to each well. After incubated for 2 min, the plates were measured and analyzed using Bio-Plex analyzer (Bio-Rad Laboratories, Inc., Hercules, CA). All incubations were done at room temperature on a microplate shaker at 800 rpm.

### 2.5. Statistical analysis

Continuous variables with normal distribution were presented as mean ± SD; otherwise, presented as median (interquartile range). One-way ANOVA was used to analyze normally distributed continuous variables and when allowed by the F value, the modified least significant difference (Fisher's PLSD) was performed to compare results and adjusted by the Bonferroni correction for multiple comparisons. For the non-normally distributed continuous variables, using the Kruskal-Wallis test to evaluate mean differences among groups, with Bonferroni post hoc analysis. Categorical variables were compared by using the chi-square test with Yates correction. Spearman correlation coefficient was used for analyzing correlations of continuous variables. Multiple linear regression analyses (forced entry method) were performed to assess the influence of variables (age, sex, BMI and other variables with P < 0.05 in the univariate model analysis) analyzed on plasma TNFRSF11B levels and AHI levels. In addition, receiving operator curves (ROC) were calculated for the prediction of OSA based on TNFRSF11B and Endocan levels and the area under curve (AUC), sensitivity, and specificity of the optimal cutoff TNFRSF11B and Endocan levels were recorded. An AUC of 0.5 indicated no predictive power, whereas an AUC of 1 indicated perfect prediction. A p-value < 0.05 was considered as statistically significant. Analyses were performed using

**Table 1**
Demographic and sleep data of 160 subjects based on severity of obstructive sleep apnea

| Parameters | Control | Mild OSA | Moderate OSA | Severe OSA | P-Value |
|---|---|---|---|---|---|
| Subjects | 40 | 40 | 40 | 40 | |
| Demographic | | | | | |
| Age (years) | 46.9 ± 15.2 | 53.2 ± 13.3* | 52.2 ± 12.8 | 55.5 ± 9.3* | 0.024 |
| Male n(%) | 27(67.5) | 29(72.5) | 37(92.5)*,† | 33(82.5) | 0.032 |
| BMI (kg/m$^2$) | 24.3 ± 3.7 | 25.2 ± 2.8 | 26.8 ± 4.0* | 27.9 ± 3.7*,† | < 0.001 |
| SBP (mm Hg) | 120.0(111.5–133.5) | 123.0(110.3–135.3) | 126.0(117.0–133.8) | 124.0(119.0–133.8) | 0.570 |
| DBP (mm Hg) | 75.5(70.0–84.5) | 72.0(70.0–84.8) | 77.5(72.3–88.5) | 81.0(76.3–88.8) | 0.056 |
| Left ventricular ejection fraction (%) | 62.5(60.0–68.0) | 65.0(60.0–68.8) | 65.5(60.3–68.8) | 63.5(58.3–68.8) | 0.711 |
| Current smoker n(%) | 13(32.5) | 14(35.0) | 15(37.5) | 19(47.5) | 0.532 |
| Coronary heart disease n(%) | 2(5.0) | 21(52.5)* | 18(45.0)* | 20(50.0)* | < 0.001 |
| Diabetes mellitus n(%) | 2(5.0) | 2(5.0) | 8(20.0) | 10(25.0) | 0.013 |
| Hypercholesterolemia n(%) | 4(10.0) | 4(10.0) | 4(10.0) | 8(20.0) | 0.433 |
| Hypertension n(%) | 11(27.5) | 18(45.0) | 24(60.0)* | 28(70.0)*,† | 0.001 |
| Sleep datas | | | | | |
| Neck circumference (cm) | 39(34–42) | 38(34–42) | 42(40–44)*,† | 43(41–44)*,† | < 0.001 |
| ESS | 0(0–4) | 4(0–9) | 8(5–12)*,† | 11(5–13)*,† | < 0.001 |
| AHI (events/h) | 2.9(1.7–3.8) | 9.8(7.1–11.0)* | 21.1(18.9-25.6)*,† | 44.7(38.3–65.7)*,†,§ | < 0.001 |
| Lowest $SaO_2$ (%) | 92(91–93) | 90(86–92)* | 87(84-89)* | 82(73-87)*,† | < 0.001 |
| Mean $SaO_2$ (%) | 97(96–98) | 96(95–97) | 95(94–96)* | 93(88-94)*,†,§ | < 0.001 |
| % of total sleep time with SpO2 < 90%(%) | 0 | 0.0(0.0–0.3) | 0.3(0.0–1.8)* | 5.1(1.3-20.6)*,†,§ | < 0.001 |
| Arousal index (events/h) | 0.5(0.0–3.1) | 4.7(0.8–11.1)* | 11.8(7.6-16.7)*,† | 12.1(6.7–40.5)*,† | < 0.001 |

Data are presented as n, mean ± SD, or median (interquartile range [IQR]) or n (%), unless otherwise stated
OSA, Obstructive sleep apnea; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; ESS, Epworth Sleepiness Scale; AHI, Apnea-hypopnea index.
* P < 0.05 versus Control.
† P < 0.05 versus Mild OSA.
§ P < 0.05 versus Moderate OSA.

SPSS version 24 software (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Clinical characteristics of the study population

The demographic and sleep data of the participants were shown in Table 1. Patients with mild OSA and severe OSA were older. BMI increased in a stepwise fashion from controls to severe OSA patients (P < 0.001). The prevalence of coronary heart disease (CHD), diabetes mellitus, hypertension in severe OSA group was higher. When sleep data were compared with controls and various degrees of severity of OSA, the ESS, AHI, the percent of total sleep time with SpO2 < 90% and arousal index were the highest in severe OSA group. Patients with severe OSA had the lowest "Lowest $SaO_2$" and "Mean $SaO_2$" compared to others. There were no differences were observed among the four groups in terms of systolic blood pressure, diastolic blood pressure, left ventricular ejection fraction, number of current smoker and the prevalence of hypercholesterolemia.

Patients with severe OSA had a significantly higher triglyceride compared to patients with mild OSA and controls (Table 2). The levels of uric acid were higher in moderate OSA group and severe OSA group. Compared with other groups, higher levels of GGT and glucose were observed in the severe OSA group. Other biological parameters were not the significant difference among the four groups.

### 3.2. Increased plasma TNFRSF11B and Endocan levels in patients with OSA

In order to identify biomarkers that can predict OSA, we evaluated twenty cytokines in patients with confirmed OSA and controls. Our study found that plasma TNFRSF11B and Endocan levels were significantly higher in the mild OSA, moderate OSA and severe OSA groups compared with the control group (Fig. 1). Remarkably, compared to the patients with moderate OSA, the plasma TNFRSF11B levels were significantly higher in the patients with severe OSA [1855.42(1451.72–2121.46) pg/mL vs. 2133.54(1841.00–2563.06) pg/mL, P < 0.05].

### 3.3. Association of TNFRSF11B level with OSA and other clinical parameters

To investigate whether the levels of TNFRSF11B were correlated with clinical and biological parameters, we used Spearman's correlation to explore the associations. As shown by correlation analyses, TNFRSF11B levels in all individuals were significantly correlated with age ($r = 0.516$, P < 0.001), AHI ($r = 0.566$, P < 0.001) and lowest $SaO_2$ ($r = -0.405$, P < 0.001). In addition, plasma TNFRSF11B levels showed a significantly strong correlation with plasma Endocan levels ($r = 0.762$, P < 0.001). (Fig. 2).

Multiple linear regression analyses were performed to examine the association of plasma TNFRSF11B levels and analyzed variables. Demographics (age, sex, BMI, presence or absence OSA, CHD, diabetes mellitus and hypertension), biological parameters (hsCRP, uric acid, creatinine and AST) and Endocan levels as covariates, found that age (Beta:0.220,95% CI: 8.357 to 21.774; P < 0.001), the presence of OSA (Beta:0.434,95% CI: 664.096 to 1076.247; P < 0.001) and the Endocan levels (Beta:0.415,95% CI: 0.188 to 0.312; P < 0.001) were independently associated with plasma TNFRSF11B levels (Table 3).

Multiple linear regression analyses were also performed to examine the association of plasma TNFRSF11B levels and the AHI. After adjustment for demographics (age, sex, BMI, presence or absence CHD, diabetes mellitus and hypertension), biological parameters (triglyceride, hsCRP, uric acid, ALT, GGT AST) and Endocan levels, plasma TNFRSF11B levels were positively associated with the AHI (p < 0.001) (Beta:0.486, 95% CI: 0.007 to 0.017; P < 0.001) (Table 4).

### 3.4. ROC curve analysis for OSA and TNFRSF11B, Endocan

ROC curves analyses were constructed to evaluate the predictive values of plasma TNFRSF11B and Endocan levels for the identification of OSA (Fig. 3). The results showed an area under the curve of 0.964 with a P value < 0.001, indicating that TNFRSF11B concentration could be a predictor of OSA. The optimal cutoff value of plasma TNFRSF11B for the identification of OSA was 1581.07 pg/mL with a corresponding sensitivity of 82.50% and specificity of 97.50%. The

**Table 2**
Biological parameters, TNFRSF11B and Endocan plasma levels of 160 subjects based on severity of obstructive sleep apnea

| Parameters | Control | Mild OSA | Moderate OSA | Severe OSA | P-Value |
|---|---|---|---|---|---|
| Subjects | 40 | 40 | 40 | 40 | / |
| Total cholesterol (mmoL/L) | 4.6(4.2–5.1) | 4.3(3.7–5.0) | 4.7(3.9–5.7) | 5.0(3.9–5.9) | 0.354 |
| Triglyceride (mmoL/L) | 1.1(0.8–1.7) | 1.3(0.9–1.8) | 1.5(0.9–1.9) | 1.7(1.3–2.1)*,† | 0.005 |
| HDL-C (mmoL/L) | 1.2(1.0–1.4) | 1.3(1.0–1.5) | 1.2(1.0–1.4) | 1.1(0.9–1.3) | 0.118 |
| LDL-C (mmoL/L) | 2.7 ± 0.9 | 2.7 ± 1.1 | 2.8 ± 1.0 | 3.1 ± 1.0 | 0.382 |
| hsCRP (mg/L) | 0.8(0.3–2.1) | 0.9(0.5–2.2) | 1.2(0.4–3.8) | 1.6(0.7–7.0) | 0.061 |
| Platelet (G/L) | 225.0(182.0–280.0) | 205.0(185.8–243.8) | 219.0(193.0–244.5) | 220(196.0–249.0) | 0.660 |
| Leukocyte (G/L) | 5.6(4.8–7.7) | 6.2(4.8–6.9) | 6.4(5.5–7.5) | 6.1(5.6–7.3) | 0.454 |
| Uric acid (umoL/L) | 342.9 ± 77.4 | 377.0 ± 96.8 | 401.5 ± 86.8* | 386.5 ± 108.3* | 0.043 |
| ALT (U/L) | 21.0(13.0–30.0) | 23.5(15.0–37.0) | 27.0(17.8–37.3) | 29.0(19.0–45.0) | 0.033 |
| GGT (U/L) | 25.5(16.3–32.8) | 32.0(20.0–43.8) | 29.0(22.0–48.5) | 48.0(27.0–72.0)* | 0.001 |
| Homocysteine (umoL/L) | 9.8(8.6–15.9) | 12.5(9.7–16.1) | 14.1(10.8–17.6) | 11.0(8.9–16.0) | 0.044 |
| Urea nitrogen (mmoL/L) | 4.5(4.0–5.5) | 5.2(4.2–6.2) | 5.1(4.4–6.9) | 5.2(4.5–6.4) | 0.133 |
| Creatinine (umoL/L) | 63.9(56.1–74.8) | 67.9(59.1–78.8) | 73.0(64.5–79.6) | 67.8(56.3–79.2) | 0.045 |
| ALP(U/L) | 74.0(64.0–90.0) | 72.5(59.3–81.0) | 74.0(64.0–90.0) | 78.0(64.0–93.0) | 0.516 |
| AST (U/L) | 20.0(17.0–24.0) | 21.0(18.0–27.0) | 24.5(18.8–30.0) | 23.0(19.0–32.0) | 0.035 |
| Creatine kinase (U/L) | 93.5(67.3–117.5) | 91.0(62.5–132.0) | 96.5(77.3–140.5) | 94.0(81.0–117.0) | 0.902 |
| Glucose (mmoL/L) | 5.1(4.9–5.4) | 5.3(5.0–5.7) | 5.4(4.8–6.0) | 5.8(5.3–7.8)*,†,§ | < 0.001 |
| TNFRSF11B(pg/mL) | 542.07(251.55–1240.06) | 2006.66(1693.52–2421.20)* | 1855.42(1451.72-2121.46)* | 2133.54(1841.00-2563.06)*,§ | < 0.001 |
| Endocan(pg/mL) | 590.41(229.53–1265.96) | 1846.10(1422.44–2747.27)* | 1634.32(1206.13-2360.87)* | 1745.54(1286.02-2691.23)* | < 0.001 |

Data are presented as n, mean ± SD, or median (interquartile range [IQR]) or n (%), unless otherwise stated.
OSA, Obstructive sleep apnea; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; hsCRP, High-sensitivity C-reactive protein; ALT, Alanine aminotransferase; GGT, γ-glutamyltransferase; ALP, Alkaline phosphatase; AST, Aspartate aminotransferase.
* P < 0.05 versus Control.
† P < 0.05 versus Mild OSA.
§ P < 0.05 versus Moderate OSA.

optimal cutoff value of plasma Endocan for the identification of OSA was 1293.94 pg/mL (sensitivity:76.70%, specificity:85.00%) and the AUC was 0.874(95% CI: 0.803–0.945, P < 0.001). Plasma TNFRSF11B showed higher discriminatory accuracy than plasma Endocan in predicting the presence of OSA (AUC, 0.964 vs 0.874, respectively).

## 4. Discussion

To our knowledge, this study was the first to investigate the plasma TNFRSF11B levels in patients with OSA. Our data showed that 1) plasma TNFRSF11B levels were significantly higher in patients with OSA. 2) Plasma TNFRSF11B levels were positively associated with the presence of OSA after adjusting confounding factors. 3) Plasma TNFRSF11B levels were independently associated with the AHI. 4) Plasma TNFRSF11B showed higher discriminatory accuracy than plasma Endocan in predicting the presence of OSA. These results indicated that plasma TNFRSF11B might be associated with vascular endothelial dysfunction, OSA severity, substantializing plasma TNFRSF11B as a potential prognostic biomarker for OSA.

In addition, our study showed that plasma TNFRSF11B levels were positively correlated with age, which was consistent with the previous studies [28]. This finding suggested that the factors were associated with aging may regulate plasma TNFRSF11B levels. We also found that the prevalence of CHD, diabetes mellitus, hypertension was higher in patients with OSA, this was consistent with previous studies [29].

CIH was the major pathophysiologic character of OSA. The cumulative effect of disorder vascular milieu resulting from CIH was thought to lead to endothelial dysfunction, which demonstrated repeatedly in patients with OSA and in animal models of intermittent hypoxia [30,31]. Furthermore, previous studies found that endothelial dysfunction appeared to be improved on cessation of the intermittent hypoxia exposure in patients with OSA [32,33]. Endothelial dysfunction, an early indicator of clinical vascular disease, played a central role in the pathogenesis of atherosclerosis [34,35]. Even in patients with OSA who were free of overt cardiovascular disease, endothelial dysfunction may underlie the development of vascular conditions such as

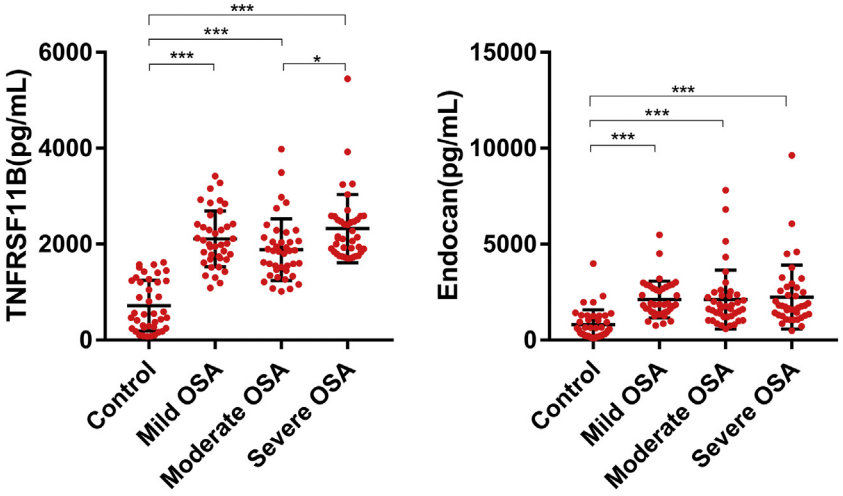


**Fig. 1.** Plasma TNFRSF11B and Endocan levels in Control, Mild OSA, Moderate OSA and Severe OSA groups. The horizontal line in the middle, top and bottom indicates the median value, the 75th and 25th percentiles, respectively; the dots represent each individual. P values < 0.05 (significant). *P < 0.05, **P < 0.01, ***P < 0.001. TNFRSF11B, Tumor necrosis factor receptor superfamily, member 11b; OSA, Obstructive sleep apnea.

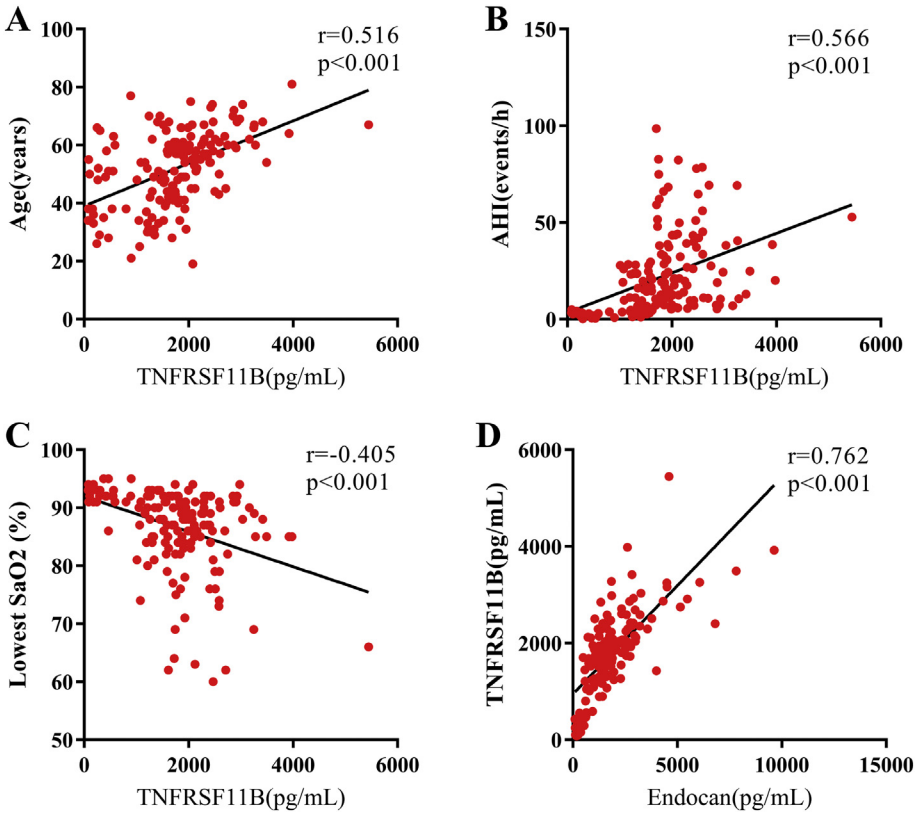


**Fig. 2.** Scatterplots of TNFRSF11B plasma levels vs age, AHI, lowest $SaO_2$ and Endocan plasma levels. r: Spearman correlation coefficients; TNFRSF11B, Tumor necrosis factor receptor superfamily, member 11b; AHI, Apnea-hypopnea index.

hypertension, ischemic stroke, and myocardial ischemia in these patients [36]. Therefore, it was extremely essential to diagnose and treat OSA in early-stage.

In general, the standard diagnostic test for OSA depends on overnight multi-channel PSG in the sleep laboratory with the primary outcome measure of AHI and several other prognostic parameters such as lowest SaO2 and the percent of total sleep time with $SpO_2 < 90\%$ [21]. However, PSG was expensive, time-consuming, labor-intensive, and in many areas, poorly accessible. It was also uncomfortable for the patients to wear the large number of sensors for data recording, which resulting in sleep disruption and underestimating the severity of OSA. These factors caused the majority persons who have OSA remains undiagnosed until complicated with serious cardiovascular and cerebrovascular complications [37]. In addition, PSG modality of testing

**Table 3**
Association between levels of TNFRSF11B and variables in univariate and multiple linear regression models

| Variables | TNFRSF11B | | | | | |
|---|---|---|---|---|---|---|
| | Univariate models | | | Multiple model ($R^2 = 0.733$,$P < 0.001$) | | |
| | Beta | 95%CI | P value | Beta | 95%CI | P value |
| Age(years) | 0.491 | 23.740 to 42.077 | < 0.001 | 0.220 | 8.357 to 21.774 | < 0.001 |
| Sex(female vs.male) | 0.022 | −289.302 to 381.438 | 0.787 | −0.104 | −414.541 to −18.909 | 0.032 |
| BMI(kg/m$^2$) | 0.211 | 13.227 to 83.682 | 0.007 | 0.032 | −15.261 to 30.465 | 0.512 |
| OSA(no vs.yes) | 0.689 | 1160.863 to 1619.979 | < 0.001 | 0.434 | 664.096 to 1076.247 | < 0.001 |
| CHD(no vs.yes) | 0.482 | 619.983 to 1114.940 | < 0.001 | 0.095 | −18.743 to 351.139 | 0.078 |
| Diabetes mellitus(no vs.yes) | 0.275 | 313.280 to 1081.914 | < 0.001 | 0.062 | −77.192 to 371.523 | 0.179 |
| Hypertension(no vs.yes) | 0.267 | 202.623 to 731.572 | 0.001 | −0.104 | −345.996 to −11.544 | 0.036 |
| Triglyceride(mmoL/L) | 0.011 | −120.828 to 138.198 | 0.895 | | | |
| hsCRP(mg/L) | 0.181 | 3.447 to 59.300 | 0.028 | 0.079 | −2.068 to 29.413 | 0.088 |
| Uric acid(umoL/L) | 0.154 | −0.035 to 2.902 | 0.056 | 0.024 | −0.761 to 1.202 | 0.657 |
| ALT(U/L) | 0.061 | −3.611 to 7.897 | 0.463 | | | |
| GGT(U/L) | 0.071 | −1.985 to 5.224 | 0.376 | | | |
| Homocysteine(umoL/L) | 0.090 | −8.774 to 30.900 | 0.272 | | | |
| Creatinine(umoL/L) | 0.159 | 0.067 to 13.134 | 0.048 | 0.059 | −1.916 to 6.626 | 0.277 |
| AST(U/L) | 0.140 | −1.802 to 23.202 | 0.093 | −0.021 | −9.969 to 6.220 | 0.648 |
| Endocan(pg/mL) | 0.720 | 0.381 to 0.517 | < 0.001 | 0.415 | 0.188 to 0.312 | < 0.001 |

Multiple linear regression model includes age, sex, BMI and other variables with $P < .05$ in univariate model analysis.
$R^2$, Adjusted $R^2$ of the multiple linear regression model; Beta, Standardized regression coefficients; CI, Confidence interals.
BMI, Body mass index; OSA, Obstructive sleep apnea; CHD, Coronary heart disease; hsCRP, Cigh-sensitivity C-reactive protein; ALT, Alanine aminotransferase; GGT, γ-glutamyltransferase; AST, Aspartate aminotransferase.

**Table 4**
Association between levels of AHI and variables in univariate and multiple linear regression models

| Variables | AHI | | | | | |
|---|---|---|---|---|---|---|
| | Univariate models | | | Multiple model ($R^2$ = 0.425, P < 0.001) | | |
| | Beta | 95%CI | P value | Beta | 95%CI | P value |
| Age(years) | 0.103 | −0.085 to 0.413 | 0.195 | −0.074 | −0.383 to 0.138 | 0.355 |
| Sex(female vs.male) | 0.122 | −1.748 to 14.083 | 0.126 | 0.028 | −5.647 to 8.528 | 0.688 |
| BMI(kg/m$^2$) | 0.451 | 1.697 to 3.226 | < 0.001 | 0.178 | 0.160 to 1.870 | 0.020 |
| CHD(no vs.yes) | 0.193 | 1.654 to 14.834 | 0.015 | −0.113 | −11.572 to 1.993 | 0.165 |
| Diabetes mellitus(no vs.yes) | 0.264 | 6.576 to 24.414 | 0.001 | 0.070 | −3.922 to 12.050 | 0.316 |
| Hypertension(no vs.yes) | 0.311 | 6.712 to 19.115 | < 0.001 | 0.179 | 1.539 to 13.402 | 0.014 |
| Triglyceride(mmoL/L) | 0.294 | 2.774 to 8.668 | < 0.001 | 0.177 | 0.651 to 6.578 | 0.017 |
| hsCRP(mg/L) | 0.192 | 0.136 to 1.517 | 0.019 | −0.014 | −0.620 to 0.505 | 0.840 |
| Uric acid(umoL/L) | 0.142 | −0.003 to 0.067 | 0.077 | −0.094 | −0.052 to 0.010 | 0.184 |
| ALT(U/L) | 0.418 | 0.234 to 0.495 | < 0.001 | 0.334 | 0.080 to 0.526 | 0.008 |
| GGT(U/L) | 0.362 | 0.115 to 0.275 | < 0.001 | 0.120 | −0.013 to 0.141 | 0.104 |
| Homocysteine(umoL/L) | −0.119 | −0.833 to 0.126 | 0.147 | | | |
| Creatinine(umoL/L) | 0.011 | −0.146 to 0.168 | 0.890 | | | |
| AST(U/L) | 0.286 | 0.245 to 0.848 | < 0.001 | −0.130 | −0.771 to 0.218 | 0.271 |
| TNFRSF11B(pg/mL) | 0.431 | 0.007 to 0.014 | < 0.001 | 0.486 | 0.007 to 0.017 | < 0.001 |
| Endocan(pg/mL) | 0.192 | 0.001 to 0.005 | 0.015 | −0.100 | −0.004 to 0.001 | 0.310 |

Multiple linear regression model includes age, sex, BMI and other variables with P < 0.05 in univariate model analysis.
$R^2$, Adjusted $R^2$ of the multiple linear regression model; Beta, Standardized regression coefficients; CI, Confidence interals.
AHI, Apnea-hypopnea index; BMI, Body mass index; CHD, Coronary heart disease; hsCRP, High-sensitivity C-reactive protein; ALT, Alanine aminotransferase; GGT, γ-glutamyltransferase; AST, Aspartate aminotransferase.

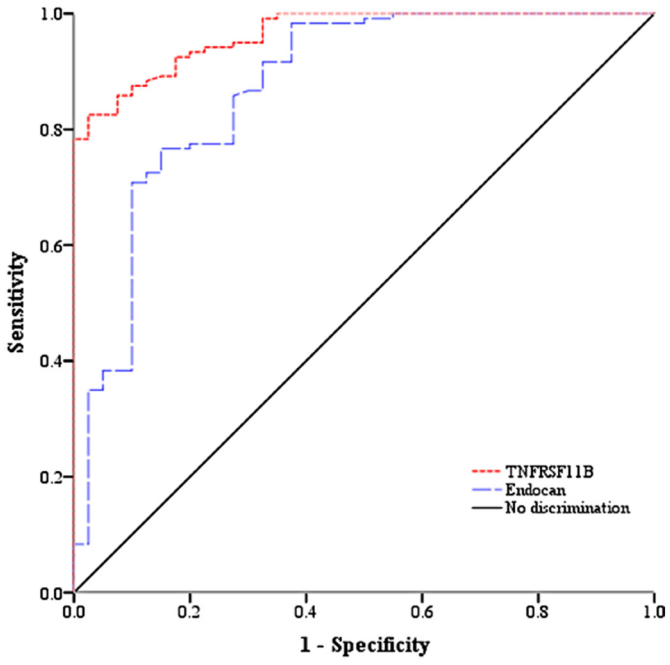


**Fig. 3.** Receiving operator curves using TNFRSF11B and Endocan plasma levels for prediction of OSA. The AUC for the plasma TNFRSF11B levels (0.964) was higher than plasma Endocan levels (0.874). TNFRSF11B, Tumor necrosis factor receptor superfamily, member 11b; OSA, Obstructive sleep apnea; AUC, Area under curve.

was not recommended for patients with heart failure and other debilitating diseases. However, because of the high prevalence of OSA and its complex complications, it was urgent to develop widely accessible tools to detect this disorder. Therefore, use of circulating biomarkers become hotspot in screening OSA.

Endocan was an endothelial-derived protein, which played an important role in endothelium-dependent pathological disorders and organ-specific inflammation [6,7]. Some evidence demonstrated that circulating Endocan levels were significant and independent indicator of OSA and its severity [8,38]. However, the recent study found that Endocan was not necessarily useful as a marker endothelial dysfunction in OSA [39]. Thus, we explored the potentially novel and potent circulating biomarker in patients with OSA.

By human Magnetic Luminex assay, we evaluated twenty cytokines in patients with confirmed OSA and controls. Cytokines were broadly chosen from endothelial activation and inflammation. Intriguingly, we found that plasma TNFRSF11B and Endocan levels were significantly higher in the OSA group compared with the control group. In addition, plasma TNFRSF11B levels were significantly higher in patients with severe OSA than patients with mild OSA. TNFRSF11B expressed in various tissues, including the bone, lung, heart, kidney, and had pleiotropic effects on bone metabolism, endocrine function and the immune system [40,41]. It had been demonstrated that circulating TNFRSF11B levels were significantly higher in patients with endothelial dysfunction as well as were associated with the prevalence and severity of coronary artery disease [42–44]. Furthermore, there was some evidence that a pathological increase of TNFRSF11B might play an important role in promoting leukocyte/endothelial cell adhesion and endothelial cells dysfunction [13,14]. Previous study had shown that TNFRSF11B as a hypoxia-inducible factor target gene capable of directing osteoblast-mediated osteoclastogenesis to regulate bone homeostasis [45,46]. In addition, plasma TNFRSF11B levels were significantly higher in mountaineers after two-week exposure to high-altitude hypoxia [47]. However, to the best of our knowledge, no previous studies have investigated the relationship between the circulating TNFRSF11B and the presence of OSA. The present study showed that the plasma TNFRSF11B levels were significantly associated with the present and severity of OSA. And intriguingly, we also found that plasma TNFRSF11B levels were strongly correlated with plasma Endocan levels. Of note, in terms of AUC, the discriminatory accuracy of plasma TNFRSF11B significantly exceeded those of plasma Endocan. These interesting findings implied that plasma TNFRSF11B levels might be related to endothelial dysfunction induced by OSA.

The strengths of the present study included strict inclusion criteria, precise and validated diagnosis of OSA and comprehensive data analyses. To avoid bias, the Luminex experiment was performed by a trained experimenter who was unaware of patients' clinical data. However, several certain limitations should be paid attention to our study. First, it was a cross-sectional study, which only shown a relationship but does not suggest causality. Second, our results didn't

elucidate the specific mechanisms of TNFRSF11B in OSA. Third, we did not evaluate the effect of CPAP therapy on plasma TNFRSF11B levels in patients with OSA.

## 5. Conclusions

In conclusion, we demonstrated that plasma TNFRSF11B levels were significantly associated with the presence of OSA and its severity. Plasma TNFRSF11B levels provided higher discriminatory accuracy levels for patients with OSA. TNFRSF11B could be a biomarker with a positive diagnostic value for premature vascular endothelial dysfunction in patients with OSA. Future studies are needed to highlight underlying mechanisms and the predictive value of TNFRSF11B for outcome in OSA patients.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.12.017.

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