

The signal axis GATA2-EDN1-AGT induced hypertension from obstructive sleep apnea-hypopnea syndrome with the clinical and animal study

Li Zhang¹, Shaokun Xu¹, Ning Zhou¹, Juanqin Shen², Jiaqing Huang³, Dongsheng Sun^{1*}

¹Department of Geriatrics, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, 310014, China

²Department of Cardiovascular Internal Medicine, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, 310014

³Department of The Second Clinic Medical College, Zhejiang Chinese Medicine University, Hangzhou, 310051, China

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ABSTRACT

Hypertension occurred in 50% obstructive sleep apnea-hypopnea syndrome (OSAHS) patients meanwhile OSAHS occurred in 30% hypertension patients. The present study aimed to explore the molecular mechanism of GATA2-EDN1-AGT induced hypertension in the development of obstructive sleep apnea-hypopnea syndrome. OSAHS patients (56 cases: 36 cases of male, 20 cases of female, 42~60 years old) were divided into two groups (case group: patients with hypertension monitored by 24 h ambulatory blood pressure and polysomnography; control group: patients without hypertension). Wistar rats were used to establish the OSAHS model (narrow pharyngeal cavity). PaO₂ and PaCO₂ of patients and rats were measured by an automatic blood gas analyzer. The profile of total protein in the OSAHS group and normal group was evaluated. Protein-protein-interaction (PPI) was carried out to show all matter proteins related. The levels of EDN-1, AGTII and atrial natriuretic peptide (ANP) in blood samples of patients and rats were analyzed by enzyme-linked immunosorbent assay (ELISA). The expression of GATA2, EDN1, endothelin-converting enzyme 1 (ECE-1) and AGT II was measured. The results showed that SaO₂ and AHI were positively associated with systolic pressure (P<0.05) in OSAHS patients. There was no correlation among other indexes (P>0.05). It was also observed that GATA2 had a strong relationship with AGT II and EDN1. The results of ELISA presented that the levels of EDN1, AGT II and ANP in the OSAHS group of human and animal models were significantly increased (P<0.05). The results of immunochemistry showed that the expression of GATA2 and AGT II in the vascular of OSAHS group was upregulated manifestly (P<0.05). It was concluded that OSAHS can induce AHI, which increases hypertension via the GATA2-EDN1-AGT II axis.

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Introduction

Sleep apnea-hypopnea syndrome (SAHS), characterized by apnea 30 times during 7 hours of sleep or apnea more than 5 times in one hour, includes three types: mix SAHS (MSAHS), central SAHS (CSAHS) and obstructive SAHS (OSAHS) (1). OSAHS is induced by abnormally anatomic structure, such as recurrent upper airway collapse, palatine tonsil enlargement and obesity. It has been found that hypertension occurred in 50% of OSAHS patients, meanwhile, OSAHS occurred in 30% of hypertension patients (2). Hypertension induced by OSAHS may be due to chronic intermittent hypoxia (CIH)-induced activation of sympathetic tone, insulin resistance and

inclined inflammation factors (3, 4). However, the molecular mechanism between hypertension and OSAHS remains unclear.

It has been reported that increased sympathetic tone can raise the level of noradrenaline which activates the renin-angiotensin-aldosterone system to prompt blood pressure by releasing endothelin (EDN1) that can re-construct the vascular smooth muscle (5). The previous study showed that the endothelial transcription factor GATA-2 (GATA-2) could promote the level of endothelin-1 (EDN1) (6). Besides, angiotensinogen (AGT), a regulator of blood pressure and electrolyte balance, has been also predicted to correlate with EDN1 and GATA2 (7, 8).

*Corresponding author. E-mail: sun_ds@21cn.com
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However, the role and molecular mechanism of the GATA2-EDN1-AGT axis in OSAHS and its relationship with hypertension are still unknown.

In the present study, we tried to elucidate the correlation between OSAHS and hypertension and the molecular mechanism for the GATA2-EDN1-AGT axis in OSAHS using both clinical study and *in vivo* animal model. This study might provide deeper insights and novel research targets for the development of OSAHS.

Materials and methods

Patients and grouping

The parent study included the part of observational clinical research, which enrolled a total of 56 OSAHS cases from the cardiovascular medicine and sleep monitoring center of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College. The diagnosis criteria of OSAHS, as well as inclusion criteria, were: 1) patients with apnea 30 times in 7 hours of sleep or apnea more than 5 times in one hour; 2) During sleep, the stopping time of oral-nasal airflow exceeded 10 s, accompanied by the amplitude of ventilation less than 50% of the base level, 3) the measurement of blood oxygen saturation decreased by 4% compared with the base value. The following patients were excluded: patients with acute coronary syndrome, severe heart failure (grade III-IV), cardiomyopathy, severe liver and kidney insufficiency, pulmonary heart disease and chronic obstructive pulmonary disease (COPD), and patients with secondary hypertension (renal parenchymal hypertension, renal vascular hypertension, primary aldosteronism and pheochromocytoma).

All patients were divided into two groups, 1) case group: patients with hypertension monitored by 24h ambulatory blood pressure and polysomnography; 2) control group: patients without hypertension. All patients agreed and signed informed consent. The present study was approved by the ethical committee of Zhejiang Provincial People's Hospital.

Animal model

Rats (male, n=20, 8 weeks, Wistar) were purchased from Changchun yisi laboratory animal technology company (Changchun, China). Rats were divided randomly into two groups after 1-week adaptation with diet balance: control group (n=10), case group

(n=10). The OSAHS model (narrow pharyngeal cavity) was built by injecting sodium hyaluronate gel to both sides of the pharyngeal cavity and root of tongue. All clinical data (oxygen saturation and blood pressure) was collected by relative machines. The model successfully constructed was confirmed according to the data.

Blood gas analysis

PaO₂ and PaCO₂ of patients and rats were measured by an automatic blood gas analyzer. Patients' apnea-hypopnea index (AHI) and night minimum oxygen saturation (SaO₂) were monitored by polysomnography. The line relationship between blood pressure and clinical indexes was measured.

Protein-protein interaction analysis

The profile of total protein in the OSAHS group and the normal group was analyzed. Protein-protein-interaction (PPI) was carried out to show all matter proteins related strictly according to the manufacturer's instruction (9).

Enzyme-linked immunosorbent assay (ELISA)

The levels of EDN-1, AGT II and atrial natriuretic peptide (ANP) in blood samples of patients and rats were analyzed by ELISA using commercial kits strictly according to the manufacturer's instruction (10-12).

Western blot

Total protein abstracted from the pulmonary vascular and coronary artery of rats was separated in PAGE gel and performed transmembrane into polyvinylidene fluoride (PVDF) membrane. After being blocked with 5% skimmed milk, all PVDF membranes were incubated in primary antibody (1:1000, GATA2, EDN1, Endothelin-converting enzyme 1 (ECE-1) and AGT II) overnight at 4°C. The samples were then incubated in secondary antibody (1:1000, 5% skimmed milk) for 2 h at 37°C. After washing in phosphate buffer solution/tween (PBST) triple times, the films were scanned using electrochemiluminescence (ECL) fluid by a UV light machine.

Immunohistochemistry

Pulmonary vascular and coronary arteries were fixed within formalin. Vascular tissue was immersed in wax and sliced into dissects. All dissects were incubated with primary antibodies (GATA2 and EDN1, 1:1000, PBST). Subsequently, dissects were incubated in secondary antibody (HRP, 1;1000, PBST) after washing in PBST triple times. Finally, dissects were dyed in DBA after washing by PBST triple times. Slices were observed under a microscope after washing by PBST triple times.

Statistical analysis

The measurement data was expressed by mean \pm SD. Comparison between two groups was performed using the Student t-test. It was considered to be statistically significant when P-value was less than 0.05. All calculations were made using SPSS 18.0.

Results and discussion

SaO₂ and AHI were positively correlated with systolic pressure

As shown in Table 1, the values of the levels of SaO₂ and AHI were significantly decreased, while levels of PaO₂, SP and DP were remarkably increased in OSAHS patients with hypertension than the non-hypertension patients (all P<0.05). Besides, SaO₂ and AHI were positively associated with systolic pressure (P<0.05, r²=0.1873) in OSAHS patients (Figure1). There was no correlation among other indexes (P>0.05).

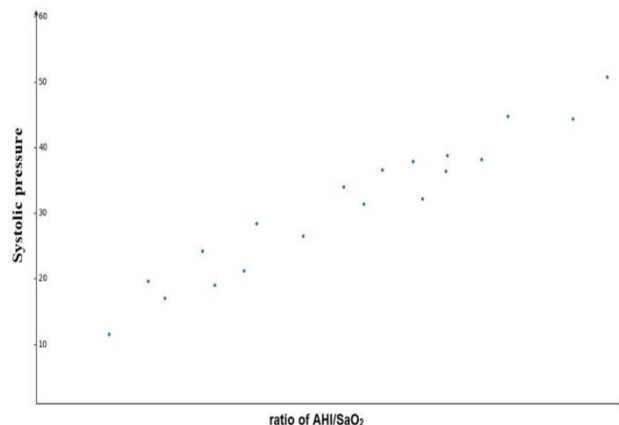


Figure 1. The relationship between AHI/SaO₂ and systolic pressure. The AHI and SaO₂ were positively correlated with systolic pressure in OSAHS patients, p<0.05, R=0.1873, n=6.

The expression of GATA2-EDN1-AGT in OSAHS patients with or without hypertension

Then the protein levels of GATA2, EDN1, ECE1 and AGT were measured. It was found that the expression of GATA2, EDN1, ECE1 and AGT was all elevated in OSAHS patients with hypertension than the non-hypertension patients (Figure 2).

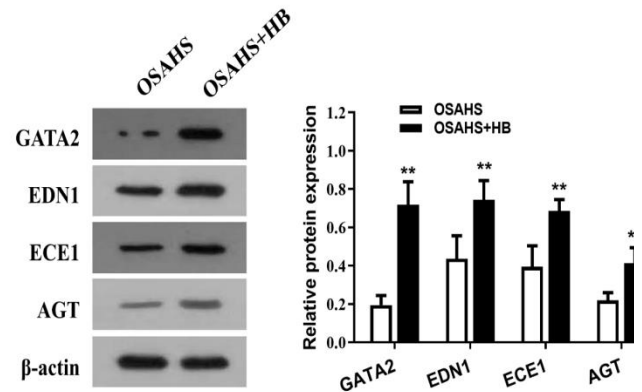


Figure 2. PPI. There is a protein-protein interaction among the EDN1, ECE1 and AGT II in OSAHS patients with or without hypertension, p<0.05, n=3.

The increase of EDN1, AGT II and ANP in the blood of OSAHS patients with hypertension

The results of ELISA presented that the levels of EDN1, AGT II and ANP in the OSAHS patients with hypertension were significantly increased than the non-hypertension patients (P<0.05) (Figure3).

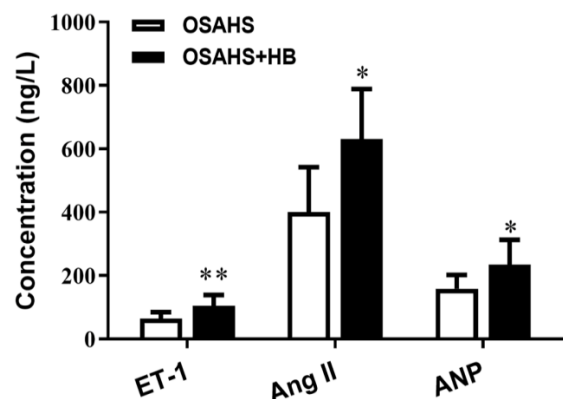


Figure 3. The concentration of EDN1, AGT II and ANP. The level of EDN1, AGT II and ANP in in OSAHS patients with or without hypertension, p<0.05.

The increase of GATA2, AGT II, ECE-1 and ANP in vascular tissue of OSAHS rats

The results of immunohistochemistry showed that the expression of GATA2 and AGT II in the vascular of OSAHS rats with hypertension was upregulated manifestly ($P < 0.05$, Figure 4). The results of western blot suggested that the level of GATA2, AGT II, the extent of cell elongation protein 1 (ECE1) and ANP was elevated significantly in the OSAHS rats with hypertension ($P < 0.05$, Figure 5).

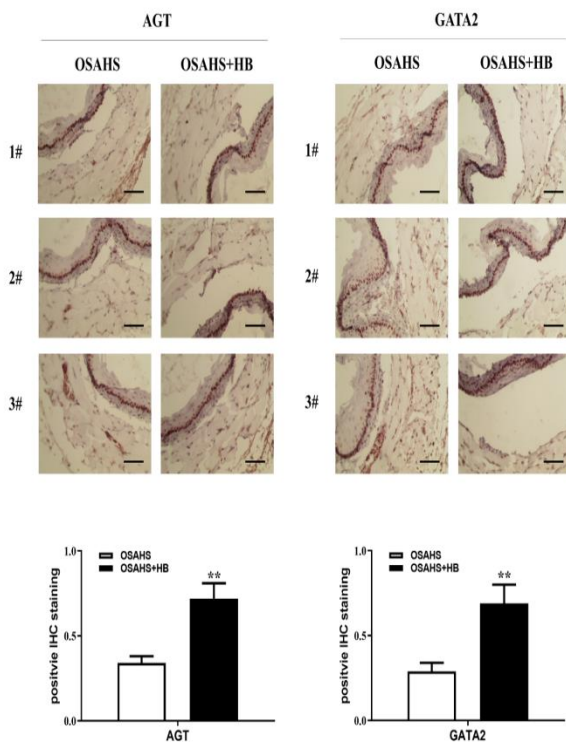


Figure 4. The level of GATA2 and AGT II in lung tissue. The level of GATA2 and AGT II in OSAHS rats with or with hypertension, $p < 0.05$, $n = 6$.

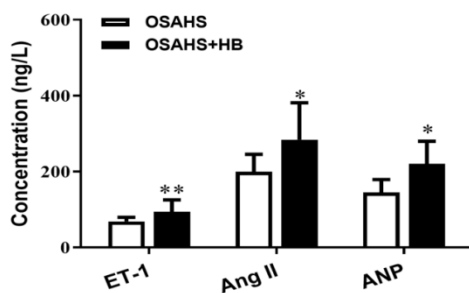


Figure 5. The level of GATA2, EDN1, ECE-1 and AGT II in lung tissue. The expression level of GATA2, EDN1, ECE-1 and AGT II in vascular of OSAHS+HT rats, $p < 0.05$, $n = 6$. compared with the OSAHS group.

OSAHS had been closely related to hypertension. It was reported that OSAHS induced hypertension by activating sympathetic tone (13), however, the molecular mechanism is unclear. This study revealed that GATA2, AGT II, EDN1 and ANP formed a network to accelerate the level of hypertension. First of all, OSAHS is often associated with sleep-disordered breathing. In our results, the SaO_2 was significantly lower in the OSAHS group. It has been reported that repeated oxygen fluctuation could induce hypertension (14), but the mechanism underlying is unknown.

Table 1. Comparison of clinical data between the two groups; Hypertension (A) and Non-Hypertension (B)

	SaO ₂ (%)	AHI	PaO ₂ (%)	SP(mm Hg)	DP(mm Hg)
A	70.3±5.1**	22.7±3.2**	83.9±4.1**	134.2±16.6**	87.5±5.4**
B	95.6±6.4	103±2.8	50.7±3.0	128.7±17.2	76.2±3.6

AHI: apnea-hypopnea index; SP: systolic pressure; DP: diastolic pressure; ** $P < 0.01$ vs non-hypertension patients.

The PPI (protein-protein interaction) showed the correlation among GATA2, AGT II and EDN1 existed. GATA2 plays an important role in cardiac muscle remodeling, which can enlarge the cardiac cell and promote the expression level of the cardiac cell (15, 16). EDN1 was considered as a key role in pulmonary arterial hypertension (17). The level of EDN1 was higher in arterial hypertension cases (18), which may suggest that EDN1 could accelerate hypertension. AGT, always considered as the trigger protein for hypertension, showed a consistency level with EDN1 (19-21). ANP drives out the sodium level to reduce hypertension. It has been reported that patients with primary hypertension have a high level of ANP (22). The evidence showed that AGT II could induce the EDN1 to accelerate hypertension, however, no direct evidence can confirm that. And the relation between GATA2 and AGT II/EDN1 was not explored yet.

In our results, the level of EDN1, AGT II and ANP in the OSAHS group was higher than in the normal group, OSAHS could induce the level of EDN1 and AGT II which were the most powerful vaso-exciters. However, the ANP increased in the OSAHS group. The function of ANP resists the effect of EDN1 and

AGT II. The phenomenon showed that organisms can keep balance via increasing the level of ANP. In the animal model, the WB results showed the GATA2, AGT II, ECE-1 and ANP were higher in the OSAHS group. ECE-1 is the rate-limiting enzyme of EDN1. The results may imply that ECE-1 can increase the level of EDN1. Based on this evidence, we may suggest that OSAHS can induce AHI which increases hypertension via GATA2-EDN1-AGT II axis.

Ethics approval and consent to participate

The ethical approval was obtained from the Ethics Committee of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College and written informed consent was obtained from all patients.

Availability of data and materials

The data are free to access to available upon request.

Competing interests

All authors declare no conflict of interest.

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Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

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