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Serum Soluble Semaphorin 4D is Associated with Left Atrial Diameter in Patients with Atrial Fibrillation

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The aim of this study was to evaluate the serum soluble semaphorin 4D (sSema4D) in patients with atrial fibrillation and to investigate the relationship of serum sSema4D with left atrial diameter (LAD).

Material/Methods: We studied a total of 113 patients who were subdivided into paroxysmal and non-paroxysmal (included persistent and permanent) atrial fibrillation groups, respectively. Another 55 subjects without atrial fibrillation were enrolled as the healthy control group. Serum levels of soluble semaphorin 4D (Sema4D) were measured in all subjects using the enzyme-labeled immunosorbent assay method. We also evaluated the coagulation parameters and left atrial diameters.

Results: Patients with paroxysmal and non-paroxysmal atrial fibrillation had significantly higher sSema4D level compared with controls (8.50±2.19 ng/mL and 9.30±2.28 ng/mL *vs.* 6.56±1.27 ng/ml, $P<0.05$). Serum sSema4D concentrations were elevated in patients with non-paroxysmal atrial fibrillation compared to those with paroxysmal atrial fibrillation ($P<0.001$). The level of sSema4D was positively correlated with LAD ($r=0.606$, $P<0.001$). Multivariate logistic regression analysis revealed that serum sSema4D, LAD, male sex, heart rate, hypertension, and coronary artery disease were associated with atrial fibrillation ($P<0.05$).

Conclusions: Serum sSema4D levels are increased in patients with atrial fibrillation and are independently associated with atrial remodeling.

MeSH Keywords: **Atrial Fibrillation • Atrial Function, Left • Semaphorins**

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Background

Atrial fibrillation (AF) is the most common type of arrhythmia in clinical practice. AF has a considerable adverse impact on the quality of life of patients and is associated with increased comorbidity and mortality [1]. At present, the underlying mechanism of AF is only partially understood. Recent studies have revealed a substantial link between AF and inflammation [2–5]. In addition, there is further evidence revealing the association between inflammatory factors and left atrial diameter (LAD) in AF [6].

Recently identified as a member of the Semaphorin protein family, semaphorin 4D (Sema4D) has been shown to play important roles in neurogenesis, immune response, angiogenesis, and tumor metastasis. Once Sema4D is activated in inflammatory cells, such as macrophages, a molecular of 120 kDa called soluble Sema4D (sSema4D) is shed from the cell membrane and can be detected in the circulating blood [7].

Recent studies have confirmed the role of sema4D as an important regulator of platelet activation and blood coagulation. Previous studies demonstrated that serum sSema4D level was associated with the atherosclerotic disease [8]. In AF, the stasis and disturbance of blood inside the atria will lead to the mural thrombosis inside cardiac chambers and may contribute to the ischemic stroke as a result of embolization. Of note, the activation of platelets is highlighted in the thrombus formation [9]. To our knowledge, the role of sSema4D in AF remains largely unknown. Thus, we performed this study to assess the association between serum sSema4D and AF in Chinese patients. Established risk factors were also measured to evaluate the role of sSema4D as an independent risk factor for AF. In addition, we also tested the hypothesis that sSema4D is linked to atrial remodeling.

Material and Methods

Study subjects

A total of 113 hospitalized patients in the second affiliated hospital of Soochow University from October, 2013 to February, 2015 were enrolled in the current study. Diagnosis of AF was confirmed according to the patient interview, past medical history and electrocardiogram. Diagnostic criteria are based on the 2013 American Heart Association (AHA) guideline for AF [10]. Paroxysmal AF was defined when recurrent AF terminates spontaneously or with intervention within 7 days of onset. Persistent AF was determined when AF continues over 7 days or requires either pharmacological or electrical cardio-conversion to restore sinus rhythm. AF, which continues for more than 6 months and/or fails to be converted into sinus rhythm for at least one attempt was defined as permanent AF. In summary, there were 56 patients with paroxysmal AF, 57 subjects with non-paroxysmal (including persistent and permanent) AF and another 55 patients without AF as a control group.

Exclusion criteria were valvular heart disease, acute coronary syndrome, acute left heart failure, infection, administration of glucocorticoids within 3 months, major trauma and operation, autoimmune disease, rheumatic activities, severe hepatic or renal dysfunction, hyperthyroidism, and hematological disease.

The current study conformed to the principles of the Declaration of Helsinki and the study protocol was approved by the ethic committee of the second affiliated hospital of Soochow University. Written inform and consent sheet were obtained from every subject.

Clinical characteristics

Clinical characteristics were obtained from all subjects on admission, such as age, gender, height, weight, heart rate, past medical history, history of cerebral embolism, CHA2DS2VASC score and medication including beta-blockers, amiodarone and anticoagulant medicines.

Blood assessment

Peripheral venous blood were obtained from each patient upon admission and stored in EDTA anticoagulation vacuum tubes. After immediate centrifugation at 3,000g for 10 minutes, serum samples were kept at –80°C. Assessment of sSema4D concentration was conducted using enzyme-labeled immunosorbent assay (ELISA) kit (Xinle Co. Ltd., Shanghai, China) according to the manufacturer's instructions. We also measured the major blood coagulation indexes upon admission. Biochemical laboratory studies were completed in the clinical lab of the hospital.

Echocardiography study

An experienced physician performed echocardiography studies in all participants within 3–5 days from admission. Images and videos were captured at left ventricular long axis and apex quad-chamber view using the Vivid 7 station (GE Company, USA). All data were recorded in a hard drive for subsequent analysis. Left atrial enlargement was determined when the left atria diameter at left supine position exceeds 40 mm.

Statistical analysis

Data analysis was performed using the SPSS 17.0 software (Chicago, IL, USA). Quantitative data was presented as x±s. For normal distributed data, student's t test was used to compare the difference between two groups. One-way ANOVA with

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Table 1. Baseline characteristics.

Variables	Paroxysmal AF (n=56)	Non-paroxysmal AF (n=57)	Non-AF (n=55)	*P* value
Age (years)	61.46±10.76	62.67±5.80	64.91±9.27	0.118
HR (bpm)	94.98±24.96	92.33±22.63	75.67±11.64	<0.001
Male gender [n (%)]	29 (51.8)	27 (47.4)	38 (69.1)	0.051
Smoking [n (%)]	15 (26.8)	19 (33.3)	22 (40.0)	0.336
Hypertension [n (%)]	30 (53.6)	30 (52.6)	53 (96.4)	<0.001
Diabetes mellitus [n (%)]	15 (26.8)	10 (17.5)	10 (18.2)	0.404
Heart valve disease [n (%)]	5 (8.9)	18 (31.6)	2 (3.6)	<0.001
COPD [n (%)]	2 (3.6)	2 (3.5)	9 (16.4)	0.014
CHD [n (%)]	9 (16.1)	5 (8.8)	27 (49.1)	<0.001
Aspirin [n (%)]	29 (51.8)	18 (31.6)	36 (65.5)	0.001
Polivy [n (%)]	6 (10.7)	9 (15.8)	18 (32.7)	0.009
Warfarin [n (%)]	8 (14.3)	17 (29.8)	0 (0)	<0.001
Beta blockers [n (%)]	28 (50.0)	26 (45.6)	39 (70.9)	0.016
Amiodarone [n (%)]	17 (30.4)	9 (15.8)	0 (0)	<0.001
CHA2DS2VASC score (scores)	1.73±1.20	1.79±0.90	–	0.775
Cerebral embolism [n (%)]	4 (7.1)	8 (14.0)	0 (0)	0.016

AF – atrial fibrillation; HR – heart rate; COPD – chronic obstructive pulmonary disease; CHD – coronary heart disease.

subsequent Turkey's post-hoc test was used to compare the difference among multiple groups. For data without normal distribution, Mann-Whitney U test was used. Categorical data was expressed as percentage and compare between groups was carried out using Kappa² test. Linear correlation analysis was performed using Spearman's test. Multivariate logistic regression using backward method was applied to evaluate the impact of different factors on the risk of AF. A two tailed P value <0.05 was considered statistically significant.

Results

Basic characteristics

Baseline characteristics of patients in each group were displayed in Table 1. There are no substantial differences among the 3 groups in age and gender ($P>0.05$). In a total of 113 patients with AF, there were 12 patients with a history of cerebral embolism, including 4 patients with paroxysmal AF and 8 patients with non-paroxysmal AF.

The level of sSema4D in patients with AF

The serum sSema4D level in patients with AF is significantly increased compared with the control group (8.50±2.19 ng/mL and 9.30±2.28 ng/mL *vs.* 6.56±1.27 ng/ml, $P=0.032$). Serum sSema4D concentrations were elevated in patients with non-paroxysmal AF compared to those with paroxysmal AF (9.30±2.28 ng/mL *vs.* 8.50±2.19 ng/ml, $P<0.001$) (Table 2).

Echocardiographic findings

The results of echocardiographic studies were summarized in table 3. Interestingly, the LADs of the AF group was significantly greater compared to the control group; subgroup analysis showed that the LADs in non-paroxysmal AF patients were significantly higher compared to those with paroxysmal AF (54.09±8.93 mm, 44.36±7.60 mm *vs.* 38.15±7.11 mm, $P<0.001$).

Blood coagulation profiles

Table 4 showed the coagulation parameters of the patients in the 3 groups. Accordingly, patients with AF had a higher coagulation state compared with the control group ($P<0.001$).

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Table 2. The sSema4D levels in different groups.

Variables	Paroxysmal AF (n=56)	Non-paroxysmal AF (n=57)	Non-AF (n=55)	*P* value
sSema4D (ng/ml)	8.50±2.19[a]	9.30±2.28[a,b]	6.56±1.27	[a]P=0.032 [b]P<0.001

[a] *P* – compared with the group of Non-AF; [b] *P* – compared with the group of Paroxysmal AF. sSema4D – sSemaphorin4D; AF – atrial fibrillation.

Table 3. Cardiac ultrasound results in different groups.

Variables	Paroxysmal AF (n=56)	Non-paroxysmal AF (n=57)	Non-AF (n=55)	*P* value
LAD (mm)	44.36±7.60	54.09±8.93	38.15±7.11	<0.001
LVEF (%)	64.12±8.06	55.97±10.78	61.38±12.61	<0.001
LVDd (mm)	46.91±7.35	50.66±6.36	44.07±8.34	<0.001
LVDs (mm)	30.67±6.89	35.15±6.80	27.64±10.58	<0.001
Long diameter of RAD (mm)	48.00±6.85	58.89±10.25	38.58±9.55	<0.001
Short diameter of RAD (mm)	35.11±6.96	44.09±9.79	28.65±7.25	<0.001
RVD [basal segment (mm)]	34.08±4.55	37.30±5.99	29.15±4.34	<0.001
Pulmonary arterial pressure (mmHg)	30.32±9.84	43.23±14.38	31.69±15.50	<0.001

AF – atrial fibrillation; LAD – left atrial diameter; LVEF – left ventricular ejection fraction; LVDd – left ventricular end-diastolic diameter; LVDs – left ventricular end-systolic diameter; RAD – right atrial diameter; RVD – right ventricular diameter.

Table 4. Comparison of main coagulation indexes between the three groups.

Variables	Paroxysmal AF (n=56)	Non-paroxysmal AF (n=57)	Non-AF (n=55)	*P* value
PT (S)	13.42±2.79	14.75±4.46	11.56±1.27	<0.001
INR	1.05±0.29	1.21±0.46	0.97±0.10	<0.001
Antithrombin III (%)	97.39±11.53	86.46±9.22	93.98±10.99	<0.001
D-two polymer (ug/ml)	0.65±1.12	0.92±0.70	0.77±0.23	<0.001

Correlation between sSema4D and left atrial diameter

Serum sSema4D was positively associated with LAD (r=0.606, P<0.001). We took LAD as the independent variable and took sSema4D as the dependent variable, then got the following related scatter diagram (Figure1).

Association between sSema4D level and AF

Multivariate logistic regression analysis showed that serum sSema4D [odds ratio (*OR*): 1.543, *95%* confidence interval (*CI*): 1.083~2.198, P=0.016], LAD (*OR:* 1.186, 95% *CI*: 1.080~1.301, P<0.001), male (*OR*: 6.368, 95% *CI*: 1.658~24.458, P=0.007), HR (*OR*: 1.050, 95% *CI*: 1.006~1.096, P=0.025), hypertension (*OR*: 27.878, 95% *CI*: 3.916~198.457, P=0.001) and CHD (*OR*: 5.967, 95% *CI:* 1.464~24.318, P=0.013) were significantly associated with AF (Table 5) after adjustment for established risk factors including age, gender, height, weight and medication including beta blockers, amiodarone and anticoagulant drugs.

Discussion

For the past decades, the underlying mechanism of AF has been extensively studied. Theories including ion channel regulation, structural or electrical remodeling and inflammation

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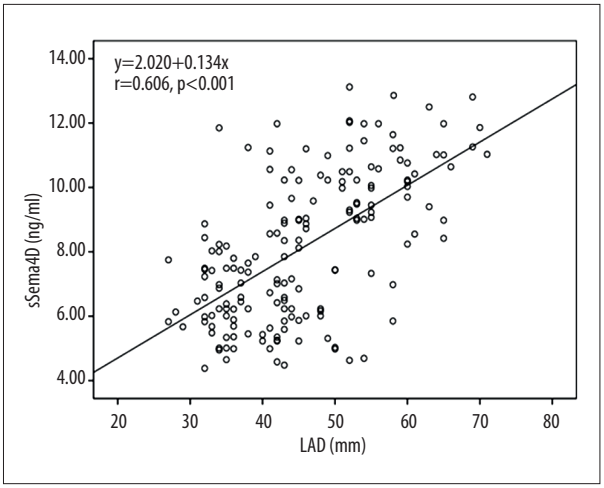

Figure 1. The related scatter diagram between sSema4D and left atrial diameter.

have been proposed as a potential basis for the onset or persistence of AF. Spectacularly, recent years have seen increasing evidence about the association between inflammation and AF. Inflammatory factors, such as HS-CRP, TNF-alpha, IL-2, IL-6, IL-8, are considered to be closely associated with the onset, persistence and recurrence of AF [11].

Sema4D, also called CD100, belongs to the class IV semaphorin protein family. Initially identified in T lymphatic cells, this molecular exerts a variety of biological functions [12]. It can bind to Plexin B1 and CD72, which are known as receptors of Sema4D, and activate signals through multiple pathways including TKR, RhoGTP, R-Ras, etc [13]. Sema4d expression has been confirmed in several cell types, such as T-cells, B-cells, NK cells, neutral granule cells, mononuclear cells, dendrite cells, macrophages, and bone marrow cells [14]. Stimulation of these cells may lead to the cleavage of Sema4D, releasing a soluble exodomain fragment called sSema4D. Subsequently, this fragment will be shed from the cell surface into the blood stream and readily detected in the circulating blood [15]. Previous studies have revealed that sSema4D retains the same bioactivity as the membrane subtype.

Activation of Sema4D is considered to play important roles in inflammation and platelet function. Our study is the first to show the association of sSema4D with AF. Increased sSema4D level is independently correlated with AF. The regulation of atrial remodeling may contribute to this link. The level of sSema4D indicates the activity of inflammatory cells. Moreover, inflammation is considered to regulate AF process. The underlying mechanism may involve inflammation, in which the activation of the inflammatory cells promotes the activation and shedding of Sema4D [16]. We hypothesized that sema4D may be the missing link between inflammation and AF.

In AF, the stasis of blood within the atria contributes to a hyper coagulate state and trend of thrombus formation. Recent studies by Zhu L. et al. demonstrated that sema4D plays an important role in platelet activation [15]. Moreover, increased cleavage and expression of sema4D have been seen in atherosclerotic disease including coronary heart disease and carotid artery stenosis. These findings suggest an association between sema4D and a thrombosis state. Accordingly, we demonstrated that sema4D is increased in AF, which is also considered to be a thrombogenic disease. Thus increased sSema4D may be a result of AF.

A growing number of studies demonstrated that atrial remodeling plays an important role in occurrence, persistence and recurrence of AF [16]. Inflammation is considered to modify the progress of the electrical, structural and neural remodeling of the atria [17]. Pathologically, atrial remodeling is reflected by gross enlargement of atrial chambers, super-microstructural change of atrial cardiomyocytes and atrial fibrosis. AF can lead to myocardial ischemia, which may further induce the unbalance between inflammatory factors and their inhibitors. Over activated inflammation may lead to the onset of AF. As a result of hypertension or aging, the intra atrial pressure

Table 5. Multiple logistic regression analysis results of atrial fibrillation.

Variables	*B*	*OR*	*95% CI*	*P*
Sema4D	0.434	1.543	1.083~2.198	0.016
LAD	0.170	1.186	1.080~1.301	<0.001
Male gender	1.851	6.368	1.658~24.458	0.007
HR	0.049	1.050	1.006~1.096	0.025
Hypertension	3.328	27.878	3.916~198.457	0.001
CHD	1.786	5.967	1.464~24.318	0.013
Constant	–11.398	0.000	–	<0.001

HR – heart rate; CHD – coronary heart disease; LAD – left atrial diameter; sSema4D – sSemaphorin4D; OR – odds ratio; CI – confidence interval.

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may increase and the atria will be enlarged, giving rise to regional pressure increment of atrial wall and result in heterogeneity of focal atrial refractory period. This is the fundamental for the generation of chaotic re-entries which can induce subsequent fibrillation activity in the atria [18]. Anatomically, atrial remodeling is a gradual process, which may progressively modify the structure of atria, causing the enlargement and dilation of atria, reflected by an elevation of LAD seen in echocardiography.

Our study demonstrated that patients with persistent AF have significantly higher LAD compared with those with paroxysmal AF or placebo, while no considerable difference was found between the last two groups. In addition, the LAD in patients with AF is positively correlated with sSema4D level. These findings suggest that sema4D may contribute to the progress of atrial remodeling. On the other hand, the persistence of AF itself may alternatively contribute to the enlargement of atria by increasing activation of sema4D.

There are several limitations in this study. The major limitation of the study is that this is a retrospective study, which included rather small sample number of the patients from a single center. In addition, we could not further completely separate the subgroup of persistent AF from permanent, and accordingly, we could not further explain whether there is a difference between these two groups. Therefore, it is needed to a larger sample and more detailed study to evaluate the expression level of sSema4D in patients with different types of AF.

Conclusions

High sSema4D level may be associated with the onset and persistence of AF. The profile of sSema4D is also correlated with atrial enlargement. In conclusion, sSema4D may regulate the occurrence and persistence of AF as well as atrial remodeling via inflammatory pathways.

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