Diagnostic Potential of Differentially Expressed Homer1 and Homer2 in Ischemic Stroke

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Key Words
Cerebral Infarction • Homer • Atherosclerosis • Diagnosis

Abstract
Background: Ischemic stroke (IS) is an extremely heterogeneous disease with variable pathogenesis. Due to the lack of early diagnostic marker, the mortality rate of IS remains high worldwide. The family of Homer plays an important role in the pathology of atherosclerotic plaque. In this study, we have investigated its expression pattern and clinical significance in IS.

Methods: RT-qPCR was performed to detect the expression of Homer1, Homer2, and Homer3.

Results: We found that the mRNA levels of Homer1 (p<0.001) and Homer2 (p<0.001), but not Homer3, in large-artery atherosclerosis (LAA) strokes were significantly upregulated than those in non-LAA strokes and controls. Multinomial logistic regression analyses showed that, although none of the Homer was associated with non-LAA strokes, higher Homer1 (adjusted OR=1.337, 95% CI: 1.227-1.458) and Homer2 (adjusted OR=1.099, 95% CI: 1.062-1.138) levels showed significant associations with increased odds of having LAA stroke, compared with the controls. The receiver operating characteristic (ROC) curves showed that the combination of Homer1 and Homer2 had a better diagnostic accuracy to differentiate LAA strokes from non-LAA strokes and controls, and the sensitivity and specificity ratios were 80.5%/90.4% and 98.0%/70.3%, respectively.

Conclusion: Our data suggested that Homer1 and Homer2 might be considered as novel diagnostic biomarkers for LAA stroke.

Introduction

Stroke is the second most common cause of mortality and the leading cause of long-term disability, with important resources consumption and socioeconomic consequences [1-3]. Stroke survivors suffer severe acquired disability, including depression, post-stroke dementia and motor dysfunction [4]. Ischemic stroke (IS) is the most common form of
stroke, accounting for approximately 87% of all strokes [5]. Although diagnostic accuracy for IS has been improved by the advents of new brain imaging technologies, none of these imaging technologies have achieved a satisfactory diagnostic value. The problems are further exacerbated by contraindications for magnetic resonance imaging (MRI) or its unavailability in small hospitals. Thus, these issues call for an easily-accessible test to further improve the convenience and correctness of diagnosis. Nowadays, the application of various blood biomarkers of cardiovascular diseases has greatly improved diagnosis and treatment strategy on myocardial infarction [6-9]. However, little progress has been made with respect to the measurement of blood biomarkers for IS. Thus, reliable blood biomarkers of IS based on disease pathophysiology are urgently needed.

Calcium (Ca\(^{2+}\)), as an important cellular messenger, is involved in many physical activities of cells and tissues, including exocytosis, apoptosis and gene regulation [10, 11]. Shanahan et al. [12] indicated that elevated Ca\(^{2+}\) has a direct effect on vascular calcification, including stimulation of osteogenic/chondrogenic differentiation, vesicle release, apoptosis, loss of inhibitors, and extracellular matrix degradation. Recent data from the Atherosclerosis Risk in Communities (ARIC) Study have shown that serum Ca\(^{2+}\) concentrations are an independent, prospective risk factor for stroke[13]. In addition, Kang et al. [14] demonstrated that hypercalcemia was positively associated with the presence of intracranial atherosclerosis. These studies have indicated that Ca\(^{2+}\) may play an important role in cardiocerebrovascular disease.

Homer proteins belong to a family of cytoplasmic adaptor proteins, including Homer1, Homer2 and Homer3 [15-17]. Homer forms a scaffold and signal transduction pathway for glutamate signalling at the post-synaptic density [18, 19]. Previous studies showed that Homer was highly expressed in the brain, skeletal muscle, and heart [19]. Current evidence supports the role for Homer in the regulation of the cytosolic Ca\(^{2+}\) homeostasis [20-23]. Jardin et al. [21] indicated that Homer played an important role in thrombin-stimulated platelet aggregation, which might be mediated by the support of agonist-induced Ca\(^{2+}\) entry. Dionisio et al. [20] demonstrated the involvement of Homer in the mechanism of regulation of Ca\(^{2+}\) entry via Cav1.2 channel. Moreover, increasing evidences prove that the store-operated Ca\(^{2+}\) entry (SOCE) is further regulated by Homer [24]. Since Homer’s important role in the regulation of the cytosolic Ca\(^{2+}\) homeostasis, more and more researchers began to investigate the role of Homer in cardiovascular disease. Of note, Homer1 was conceptualized as having a diagnostic potential for coronary artery disease, however, the clinicopathologic and diagnostic significance of Homer in IS has not been reported [25].

In the present study, we investigated the levels of Homer1, Homer2, and Homer3 in IS patients, then analyzed the relationships between Homer expression and clinical characteristics, and evaluated the prognostic and diagnostic value of Homer. In conclusion, our aim was to provide an important basis for prognosis and diagnosis in IS.

**Materials and Methods**

**Experimental subjects**

We recruited 210 patients (135 men and 75 women, mean age, 71.9±11.5) who were diagnosed as IS between September 2015 and June 2016 at Zhongnan Hospital of Wuhan University, Wuhan, China. Patients were included in this study if they had experienced their first ischemic stroke within the preceding 2 days. The diagnosis of IS in all patients was confirmed by brain computed tomography (CT) and/or MRI. Subtypes of IS were determined according to the classification of Trial of Org 10172 in Acute Stroke Treatment (TOAST) [26]. IS patients were classified into three groups: large-artery atherosclerosis (LAA, 62 men and 38 women, mean age 72.2±11.1), small vessel occlusion (SVO, 46 men and 22 women, mean age 71.6±12.0) and cardioembolism (CE, 27 men and 15 women, mean age 72.5±11.9). We also collected 226 controls (150 men and 76 women, mean age, 71.1±8.3) who came from the Physical Examination Center. The exclusion criteria included acute or chronic renal dialysis; malignant tumors; autoimmune disease; acute or chronic infections or inflammatory diseases.
Demographic and clinical information was collected and included the following: age, gender, height, weight, hypertension (systolic/diastolic blood pressure ≥140/90 mmHg or prior diagnosis of hypertension or on antihypertensive medication), diabetes mellitus (fasting blood sugar >126 mg/dL or prior diagnosis of diabetes or on glucose-lowering medication), hypercholesterolemia (fasting cholesterol level ≥240 mg/dL or prior diagnosis of dyslipidemia or on lipid-lowering medication), alcoholism and current smoker. Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared. Laboratory information, such as HbA1c, total cholesterol (TC), triglyceride (TG), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), and lipoprotein (a) [Lp (a)], was also gathered.

**Ethical approval**

Specimens and clinical materials were collected after obtaining the informed consent of patients in accordance with institutional ethical guidelines, which was all approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (Wuhan, China).

**RNA extraction and reverse transcription**

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from peripheral blood leukocytes. The concentration and purity of RNA were measured by Nanodrop ND2000 (Thermo Scientific Inc., Waltham, MA, USA). RNA was reverse transcribed to cDNA by using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan), following: 42°C for 2 min, and then 37°C for 15 min, 85°C for 5 sec.

**Real-time PCR analysis**

Expression levels of Homer1, Homer2 and Homer3 were performed on the Bio-Rad CFX96 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR-Green I Premix EXTaq according to the manufacturer’s instructions. In order to normalize the results for the qPCR, expression of GAPDH was used. The synthesized primers were as follows: GAPDH (forward: 5’-AGAGACGTGGGCTCATTG-3’ and reverse: 5’-CGAGGAGGATCGTGATGATG-3’); Homer1 (forward: 5’-GATCCCTGCTAGCCTTCT-3’ and reverse: 5’-GAGGAGGCACAAAGG-3’); Homer2 (forward: 5’-TACGGTTTCTACTCTAT-3’ and reverse: 5’-CCTGCGTCTTGTCTT-G-3’); Homer3 (forward: 5’-TGACTACCTGCTCCTATTT-3’ and reverse: 5’-GGAACCCTGAGCAAAG-3’). The total reaction volume was 20 μL, which contained 2 μL cDNA, 10 μL SYBR-Green Supermix, 1.6 μL gene-specific forward and reverse primers, and 6.4 μL nuclease-free water. The reactions started at 95°C for 5 min, followed by 45 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. All experiments were carried out in duplicate for each data point. Relative gene expression levels were calculated using the comparative Ct method formula 2−ΔΔCt.

**Statistical analysis**

All statistical analyses were performed using SPSS version 23.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Normally distributed data were presented as mean±standard deviation (M±SD). Skewed data were described by the median and inter-quartile range. P<0.05 was considered to be statistically significant. The Shapiro-Wilk test was used to check the normality of the distribution. The differences between normally distributed numeric variables were evaluated by Student’s t-test, whereas non-normally distributed variables were analyzed by Mann-Whitney U-test. One-way ANOVA was used for the comparison among multiple groups if the variance was homogeneous, while non-normally distributed variables were evaluated by Kruskal-Wallis variance analysis. The categorical variables were analyzed using Chi-square test. Associations between IS and levels of Homer were calculated through binary logistic regression analysis, taking IS as a dependent variable. Odds ratios with 95% confidence intervals were computed. To calculate the points of estimates across stroke subtypes, multinomial logistic regression analysis models were used taking a polychotomous category of stroke subtypes (control group as a reference) as a dependent variable. Each Homer was entered separately into logistic regression models. Both multivariable logistic models were performed by incorporating age, sex, BMI, hypertension, smoking, alcoholism, LDL-C, HDL-C, TG, TC, Lp(a) and HbA1c. Finally, the receiver operating characteristic (ROC) curve analysis was performed to estimate the diagnostic values.
Results

Patient characteristics

The main demographic and clinical characteristics of the studied subjects were illustrated in Table 1. No difference was observed in regard to important risk factors including age, gender, BMI, TG, smoking, alcoholism, diabetes, hypercholesterolemia, LDL-C and Lp(a) in the four groups. There was a significant difference in hypertension, HDL-C, TC and HbA1c among the groups.

Homer1 and Homer2 expression in peripheral blood leukocytes are upregulated in LAA patients

To observe the value of Homer1, Homer2 and Homer3 as biomarkers, the levels of target mRNAs in all subjects were measured by RT-qPCR. The results showed that the mRNA levels of Homer1 and Homer2 were significantly upregulated in IS patients compared to the control group (Homer1: P<0.001, Fig. 1 A; Homer2: P<0.001, Fig. 1 B), but not Homer3 (P=0.258, Fig. 1 C). Further research indicated that the mRNA levels of Homer1 and Homer2 in the LAA group were higher than that in CE, SVO, and the control groups (Homer1: LAA vs CE, P<0.001; LAA vs SVO, P<0.001; LAA vs the controls, P<0.001, Fig. 1 D; Homer2: LAA vs CE, P<0.001; LAA vs SVO, P<0.001; LAA vs the controls, P<0.001, Fig. 1 E). However, upon comparison of the levels in the other three groups (CE, SVO and the controls), no marked

Fig. 1. The expression levels of Homer in peripheral blood leukocytes among subgroups. (A). Homer1 levels in ischemic stroke (IS) were significantly higher than that in the controls. (B). Homer2 levels in IS were significantly higher than that in the controls. (C). No significant difference of Homer3 were observed between IS and the controls. (D). Homer1 levels in large-artery atherosclerosis (LAA) were significantly higher than that in cardioembolism (CE), small vessel occlusion (SVO) and the control groups. No differences were observed among CE, SVO and the control groups. (E). Homer2 levels in LAA were significantly higher than that in CE, SVO and the control groups. No differences were observed among CE, SVO and the control groups. (F). No significant difference of Homer3 were observed among these groups. Results were expressed as mean±SD. The data analyzed using Student’s t-test and One way ANOVA. -lg2 ΔCt values for each individual were used to create each figure. * P < 0.001.
Table 1. Characteristics of the studied subjects. Data are mean±SD or percentage or median (25% percentiles, 75% percentiles). Abbreviations: *Chi-square test; †Oneway ANOVA; ‡Kruskal-Wallis; LAA: large-artery atherosclerosis; CE: cardioembolism; SVO: small vessel occlusion; BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; Lp(a): lipoprotein (a). P<0.05 was considered statistically significant

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>LAA (n = 100)</th>
<th>CE (n = 42)</th>
<th>SVO (n = 68)</th>
<th>Control (n = 226)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>62 (62.0%)</td>
<td>27 (64.3%)</td>
<td>46 (67.6%)</td>
<td>150 (66.4%)</td>
<td>0.856†</td>
</tr>
<tr>
<td>Age, years</td>
<td>72.2±11.1</td>
<td>72.5±11.9</td>
<td>71±12.0</td>
<td>71±8.3</td>
<td>0.371†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1±2.5</td>
<td>23.6±2.6</td>
<td>24.0±2.7</td>
<td>23.8±2.2</td>
<td>0.570‡</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>64 (64.0%)</td>
<td>30 (71.4%)</td>
<td>47 (69.1%)</td>
<td>98 (43.4%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diabetes</td>
<td>29 (29.0%)</td>
<td>14 (33.3%)</td>
<td>19 (27.9%)</td>
<td>57 (25.2%)</td>
<td>0.702†</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>32 (32.0%)</td>
<td>11 (26.2%)</td>
<td>18 (26.5%)</td>
<td>47 (20.8%)</td>
<td>0.182*</td>
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<tr>
<td>Smoking</td>
<td>31 (31.0%)</td>
<td>9 (21.4%)</td>
<td>16 (23.5%)</td>
<td>43 (19.0%)</td>
<td>0.126*</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>20 (20.0%)</td>
<td>10 (23.8%)</td>
<td>15 (22.1%)</td>
<td>27 (11.9%)</td>
<td>0.059#</td>
</tr>
<tr>
<td><strong>Laboratory variables</strong></td>
<td></td>
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</tr>
<tr>
<td>TC, mg/dL</td>
<td>172±47.5</td>
<td>158.9±43.4</td>
<td>161.6±53.9</td>
<td>152±25.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>119 (84, 193)</td>
<td>126 (72, 189)</td>
<td>116 (97, 183)</td>
<td>123 (96, 151)</td>
<td>0.083*</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>106±34.2</td>
<td>103±35.4</td>
<td>95±31.0</td>
<td>101±21.8</td>
<td>0.115*</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>44±11.9</td>
<td>43.0±10.5</td>
<td>41±11.0</td>
<td>55±15.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>155 (98, 303)</td>
<td>193 (112, 364)</td>
<td>206 (145, 303)</td>
<td>183 (124, 302)</td>
<td>0.605*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>6.7±1.4</td>
<td>6.5±1.3</td>
<td>6.6±1.4</td>
<td>6.2±1.2</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

Table 2. The proportion of each stroke subtype according to the quartiles of Homer1. Abbreviation: *Chi-square test; LAA: large-artery atherosclerosis; CE: cardioembolism; SVO: small vessel occlusion. -lg2−ΔCt values for each individual were used. P<0.05 was considered statistically significant

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>Quartiles of Homer1, range, relative expression (-log), N=436</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st, &lt;2.051</td>
</tr>
<tr>
<td>LAA</td>
<td>64 (58.7%)</td>
</tr>
<tr>
<td>CE</td>
<td>6 (5.5%)</td>
</tr>
<tr>
<td>SVO</td>
<td>16 (14.7%)</td>
</tr>
<tr>
<td>Control</td>
<td>23 (21.1%)</td>
</tr>
</tbody>
</table>

Table 3. The proportion of each stroke subtype according to the quartiles of Homer2. Abbreviation: *Chi-square test; LAA: large-artery atherosclerosis; CE: cardioembolism; SVO: small vessel occlusion. -lg2−ΔCt values for each individual were used. P<0.05 was considered statistically significant

<table>
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<tr>
<th>Subject groups</th>
<th>Quartiles of Homer2, range, relative expression (-log), N=436</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1st, &lt;1.805</td>
</tr>
<tr>
<td>LAA</td>
<td>52 (47.7%)</td>
</tr>
<tr>
<td>CE</td>
<td>9 (8.3%)</td>
</tr>
<tr>
<td>SVO</td>
<td>10 (9.1%)</td>
</tr>
<tr>
<td>Control</td>
<td>38 (34.9%)</td>
</tr>
</tbody>
</table>

Table 4. The proportion of each stroke subtype according to the quartiles of Homer3. Abbreviation: *Chi-square test; LAA: large-artery atherosclerosis; CE: cardioembolism; SVO: small vessel occlusion. -lg2−ΔCt values for each individual were used. P<0.05 was considered statistically significant

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>Quartiles of Homer3, range, relative expression (-log), N=436</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st, &lt;1.729</td>
</tr>
<tr>
<td>LAA</td>
<td>28 (25.7%)</td>
</tr>
<tr>
<td>CE</td>
<td>8 (7.3%)</td>
</tr>
<tr>
<td>SVO</td>
<td>18 (16.5%)</td>
</tr>
<tr>
<td>Control</td>
<td>55 (50.5%)</td>
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</table>
differences were found. Moreover, no differences were observed in regard to the mRNA levels of Homer3 in the four groups (Fig. 1 F). Of note, when we divided the levels of each
Homer into quartiles, we also found that the proportion of LAA strokes increased according to the elevated level of Homer1 (P<0.001, Table 2) and Homer2 (P<0.001, Table 3), but not Homer3 (P=0.581, Table 4).

In univariate analysis, increasing Homer1 (P<0.001, OR=1.209, 95% CI: 1.145-1.276) and Homer2 (P<0.001, OR=1.063, 95% CI: 1.039-1.089) levels showed significant associations with IS, however, Homer3 (P=0.325, OR=1.012, 95% CI: 0.988-1.036) did not. After adjusting for relevant clinical and laboratory variables, elevated Homer1 (P<0.001, adjusted OR=1.168, 95% CI: 1.090-1.252) and Homer2 (P<0.001, adjusted OR=1.058, 95% CI: 1.027,1.091) levels remained significant with regard to increased odds of having IS. However, Homer3 (P=0.736, adjusted OR=0.994, 95% CI: 0.962-1.027) failed to show significant association with IS in multivariable analyses.

When IS subtypes were considered in multinomial logistic regression models, none of the Homer showed a significant association with non-LAA strokes (CE and SVO). However, higher Homer1 (P<0.001, adjusted OR=1.337, 95% CI: 1.227-1.458) and Homer2 (P<0.001, adjusted OR=1.099, 95% CI: 1.062-1.138) levels were significantly associated with increased odds of having LAA stroke, compared with the controls (Table 5). Homer3 showed no association with either LAA strokes (P=0.672) or non-LAA strokes (CE: P=0.341, SVO: P=0.655).

### Stability detection of Homer1, Homer2 and Homer3

Our study amplified Homer1, Homer2 and Homer3 in peripheral blood leukocytes of IS patients for the first time. Because a proportion of patients with IS enrolled in our study received a bolus of 5000 IU of heparin, we detected the stability of Homer1, Homer2 and Homer3. We collected whole blood from 5 healthy human (3 men and 2 women) and added heparin (1 IU/mL of blood) to each sample, then they were stored at 37 °C for 0, 10, 30 and 60 min. The expression levels of Homer1, Homer2 and Homer3 were assessed by RT-qPCR. The results showed that heparin had minimal effects on the quantification of Homer1, Homer2 and Homer3 (Fig. 2).

### Correlation between Homer and clinical variables in IS

We detected the correlation between the expression levels of Homer1 and Homer2 and clinical parameters in the 210 IS patients. As shown in Table 6, no significant associations
were found in regard to gender, age, BMI, smoking, alcoholism, hypertension, diabetes, hypercholesterolemia, HbA1c and other biochemical indices.

**Diagnostic value of Homer in peripheral blood leukocytes**

To assess whether Homer1, Homer2 and Homer3 could be used as potential diagnostic markers for IS, ROC was constructed using 4 models: LAA vs the controls, CE vs the controls, SVO vs the controls and LAA vs non-LAA (Table 7). It was revealed that Homer1 and Homer2 were potential markers for discriminating LAA patients from the controls (Fig. 3 A). Combination of Homer1 and Homer2 possessed a remarkable ability for discrimination between LAA patients and controls (AUC=0.927, 95% CI: 0.900-0.954) (Fig. 3 B). However, compared with the group of LAA vs the controls, the diagnostic value of Homer in the CE vs the controls and SVO vs the controls groups was not obvious (Fig. 3 C-D). Moreover, we also verified the discriminative ability of Homer in identifying LAA patients from non-LAA patients. As shown in Figure 3E, Homer1 and Homer2 had moderate abilities for discrimination between LAA and non-LAA patients (Fig. 3 E). Combination of Homer1 and Homer2 possessed a good sensitivity of 98.0% (AUC=0.858, 95% CI: 0.804-0.911) (Fig. 3 F) for discrimination between LAA and non-LAA patients.
Discussion

IS is an extremely heterogeneous disease with variable pathogenesis. Of note, IS uniformly occurs when brain-supplying arteries are occluded by drifting embolus or by local thrombus formation. However, causative pathogenesis and physiological mechanisms for formation of thrombus differ across stroke subtypes. LAA occurs in accordance with rupture of atherosclerotic plaques at the site of arterial occlusion, which may have direct relationship to atherosclerosis [27]. On the contrary, other stroke subtypes are defined to develop without significant relationship to plaque pathology. In this context, it may be extrapolated from our result that stroke subtypes should be one of the important considerations in investigating study of the value of stroke biomarkers linked to the rupture of atherosclerotic plaques.

Homer is a family of three adaptor proteins, with a protein-binding domain structure consisting of an N-terminal class II Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) homology 1 (EVH1) [28]. The EVH1 domain binds to multiple ligands, including G protein-coupled receptors (GPCRs), ryanodine receptors, transient receptor potential channels (TRPC) and inositol 1,4,5-triphosphate receptors, which has been reported to play different roles in Ca\(^{2+}\) signalling [22, 29]. For example, Homer1 has been reported to be essential to regulate the TRPC in the closed configuration and resting state. Homer1 has also been hypothesized to associate with Orai1 and STIM1 in human platelets [22]. Homer2 plays a crucial role in the regulation of GPCRs, then, results in attenuation of the Ca\(^{2+}\) signal intensity [30]. Previous studies have demonstrated that Homer may play an important role in the regulation of the cytosolic Ca\(^{2+}\) homeostasis, and more and more researchers began to investigate the role of Homer in cardiovascular disease [25]. However, the diagnostic significance of Homer in IS has not been reported.

In the present study, we for the first time investigated the clinical value of Homer in IS patients. We found that Homer1 and Homer2 were significantly upregulated in IS patients after adjustment for relevant covariates. However, no significant difference was found in Homer3. When further considering the subtypes of ischemic strokes, we documented that Homer1 and Homer2 had differential association patterns among stroke subtypes. LAA stroke was associated with higher Homer1 and Homer2 levels, while for non-LAA stroke subtypes, such as CE and SVO, none of Homer showed significant associations. Recently, Jing et al. [25] found that the mRNA level of Homer1 in peripheral blood leukocytes in CAD patients was significantly higher than that in the controls, which indicated that Homer1 may be a potential and important participant of atherosclerotic plaque development and eventually rupture in coronary artery disease. Considering the role of atherosclerotic plaques in development of LAA stroke and their association with Homer, we may suppose that Homer1 and Homer2 are important participants in early atherosclerosis, consequently, IS.

Heparin is commonly used as an anticoagulant in cardiovascular diagnostics and interventions. Boeckel et al. [31] indicated that heparin can inhibit RNA quantification \textit{in vitro} by interfering with the DNA polymerase used in the qPCR reaction. In the present study, we detected the expression of Homer in peripheral blood leukocytes added with heparin to assess the stability of Homer in IS patients. No significant difference was found in the process. The possible explanations for the discrepancy include the following. First, the concentration of heparin in IS patient blood is significantly lower than in heparinized blood collection tubes (11–32 IU/mL) [32]. Second, we have detected mRNAs, whereas Boeckel et al. [31] have detected microRNAs, which may be more sensitive to interference by heparin than mRNAs.

For the first time, we detected the expression of Homer in peripheral blood leukocytes to analysis the diagnostic value. The area under the ROC showed that Homer1 and Homer2 had a better diagnostic value to differentiate LAA strokes from non-LAA strokes and controls, but the diagnostic value of Homer in the other two ischemic stroke subtypes were not obvious. Further analysis showed that the combination of Homer1 and Homer2 had a better diagnostic accuracy. Thus, Homer1 and Homer2 may represent promising targets for LAA stroke diagnosis.
This study has several limitations. First, Homer, also known as a family of skeleton proteins, closely bind to the cell membrane, and meanwhile, there is no reliable reagent directly to detect Homer proteins in plasma. Thus, we failed to detect the levels of Homer proteins in plasma. Second, Homer were measured from post-stroke samples in our study. Third, this retrospective study was conducted in a single hospital, thereby being prone to selection bias.

In conclusion, the present study provides insights into the expression levels of Homer in IS patients for the first time. We elaborated that the mRNA levels of Homer1 and Homer2 in the peripheral blood leukocytes were significantly higher in LAA strokes than in non-LAA strokes and controls, and the combination of Homer1 and Homer2 had a significantly diagnostic value. These findings suggested for the first time that the expression of Homer1 and Homer2 could be used as novel diagnostic biomarkers for LAA stroke. Future studies are warranted to further evaluate biomarker potentials and to assess the pathomechanism responsible for these increases.

Acknowledgments

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Disclosure Statement

The authors have declared that no conflict of interest.

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