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Plasma Fibroblast Growth Factor 23 Concentration Is Associated with Intracranial Cerebral Atherosclerosis in Acute Ischemic Stroke Patients

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Background and Purpose Fibroblast growth factor 23 (FGF23) is associated with atherosclerosis via nitric-oxide-associated endothelial dysfunction and calcium-phosphate-related bone mineralization. This study aimed to determine the association of the plasma FGF23 concentration with intracranial cerebral atherosclerosis (ICAS) and extracranial cerebral atherosclerosis (ECAS).

Methods We prospectively enrolled 262 first-ever ischemic stroke patients in whom brain magnetic resonance was performed and a blood sample acquired within 24 h after admission. Plasma FGF23 concentrations were measured using an enzyme-linked immunosorbent assay. The presence of ICAS or ECAS was defined as a ≥50% decrease in arterial diameter in magnetic resonance angiography. The burden of cerebral atherosclerosis was calculated by adding the total number of vessels defined as ICAS or ECAS.

Results Our study population included 152 (58.0%) males. The mean age was 64.7 years, and the plasma FGF23 concentration was 347.5±549.6 pg/mL (mean±SD). ICAS only, ECAS only, and both ICAS and ECAS were present in 31.2% (n=82), 4.9% (n=13), and 6.8% (n=18) of the subjects, respectively. In multivariate binary and ordinal logistic analyses, after adjusting for sex, age, and variables for which p<0.1 in the univariate analysis, the plasma FGF23 concentration (per 100 pg/mL) was positively correlated with the presence of ICAS [odds ratio (OR)=1.07, 95% CI=1.00−1.15, p=0.039], burden of ICAS (OR=1.09, 95% CI=1.04−1.15, p=0.001), and burden of ECAS (OR=1.06, 95% CI=1.00−1.12, p=0.038), but it was not significantly related to the presence of ECAS (OR=1.05, 95% CI=0.99−1.12, p=0.073).

Conclusions The plasma FGF23 may be a potential biomarker for cerebral atherosclerosis, particularly the presence and burden of ICAS in stroke patients.

Key Words fibroblast growth factor 23, Klotho, cerebral atherosclerosis, intracranial atherosclerosis.

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INTRODUCTION

Intracranial cerebral atherosclerosis (ICAS) is a major risk factor for cerebrovascular disease, particularly in Asians. ICAS is also associated with recurrent stroke, stroke-related morbidity, and future mortality.[1] The importance of ICAS for stroke patients and the associated factors and risk factors remain unclear, although they have been suggested to be aging, racial differences (mainly Asian), hypertension, diabetes mellitus, smoking, obstructive sleep apnea, and obesity.[2-4] Thus, further evaluations to find the factors that explain ICAS are necessary.

Fibroblast growth factor 23 (FGF23) is an endocrine FGF and phosphaturic hormone

mainly formed by osteoblasts and osteocytes.[5] In harmony with Klotho (a single-pass transmembrane protein expressed in renal tubules), as an obligate coreceptor to bind and activate FGF receptors, FGF23 is activated during bone mineralization and turnover defects.[6] In addition, kidney dysfunction, adipose tissue inflammation, and vitamin D dysregulation are involved in systemic FGF23 regulation.[7] FGF23 is also involved in systemic atherosclerosis via nitric-oxide-associated endothelial dysfunction and calcium-phosphate-related bone mineralization.[8] Previous studies have found increased FGF23 to be associated with subclinical atherosclerosis and an increased left ventricular mass.[9-11] Furthermore, FGF23 is an independent predictor of cardiovascular events in the general population.[12]

Major features of ICAS include atherosis caused by cholesterol deposition and inflammation and sclerosis secondary to endothelial dysfunction, leading to arterial stiffness.[1] Because FGF23 is also involved in the development of atherosclerosis and endothelial dysfunction,[9-11,13] this growth factor might also be associated with the atherosclerosis of intracranial vessels. We hypothesized that a higher circulating FGF23 concentration is associated with the presence and burden of cerebral atherosclerosis in patients with acute ischemic stroke.

METHODS

Subjects

We prospectively registered 262 patients with first-ever ischemic stroke who were admitted to our institution between June 2014 and May 2016 within 7 days after symptom onset and had a stroke subtype classified as large-artery atherosclerosis, cardioembolism, or small-vessel occlusion. Standard protocols for the stroke registry were applied to all patients, which included a chest X-ray; 12-lead electrocardiography; routine blood tests at admission (white blood cell count, WBC) and creatinine) and after a 12-h fast {vitamin D [25(OH)D], fasting glucose, HbA1c, triglyceride, total cholesterol, low-density lipoprotein, total calcium, phosphate, albumin, alkaline phosphatase, uric acid, and CRP}; brain imaging with CT and/or MRI; and vascular imaging with CT angiography, magnetic resonance angiography (MRA), or digital subtraction angiography.[14,15] Patients who did not agree to participate in the study, refused to provide blood samples, or who had a history of cancer, autoimmune disease, or bone fractures during the previous 2 months were excluded.

Risk factors were defined as in a previous study and the Supplementary Materials (in the online-only Data Supplement).[16] The stroke subtype was classified using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system.[17] Briefly, patients with potential cardiac emboli were classified as the cardioembolic subtype. If a patient exhibited substantial stenosis of the relevant artery, they were classified as the large-artery atherosclerosis subtype. The small-vessel occlusion subtype was defined if all of the following criteria were met: clinical manifestation of classical lacunar syndrome, relevant subcortical or brainstem lesion, infarction smaller than 15 mm, no significant stenosis in the relevant artery, and no potential cardiac embolism.[18] The Fazekas scoring system was used to assess white-matter hyperintensities (WMHs) in MRI FLAIR.[19] A Fazekas score of ≥2 in the periventricular or deep white matter was defined as high-grade WMH. The renal function was investigated using the estimated glomerular filtration rate according to the Modification of Diet in Renal Disease study equation.[20] This study was approved by our Institutional Review Board (approval no. ECT 2014-04-023), and we obtained informed consent from all participants and their next of kin.

MRI protocol and definition of vascular stenosis

Every patient enrolled in this study underwent intracranial and extracranial MRA. All MR images were obtained using a 3.0-T imaging system (Magnetom Verio, Siemens Healthcare, Erlangen, Germany). Three-dimensional time-of-flight sequences were used to evaluate the intracranial arteries (intracranial internal carotid artery, middle cerebral artery, anterior cerebral artery, posterior cerebral artery, distal vertebral artery, and basilar artery) and the extracranial arteries (extracranial internal carotid artery and common carotid artery). Extracranial cerebral atherosclerosis (ECAS) was not assessed in the extracranial vertebral arteries because it is difficult to distinguish hypoplasia of the vertebral artery from significant stenosis.[3]

The method used in this study to evaluate stenosis has been described previously,[21] and >50% stenosis was defined as ICAS.[22] ECAS was assessed using the criteria from the North American Symptomatic Carotid Endarterectomy Trial, with its presence defined as >50% stenosis at the bifurcation of the bilateral carotid artery.[23] The most serious lesion was chosen when multiple stenotic lesions were present.[22,24] The total number of vessels with ICAS was considered as the ICAS burden (ranging from 0 to 11), while the total number of vessels with ECAS was considered the ECAS burden (ranging from 0 to 4).[24] Two neurologists blinded to clinical information (Y.C. and T.J.S.) independently assessed the presence of ICAS. The kappa value for interobserver agreement was 0.936 for the presence of ICAS. Any initial disagreements in the neurologist assessments of ICAS were resolved by discussion.

Measurement of plasma FGF23 concentrations

To measure the plasma FGF23 concentration, a blood sample were collected after a 12-h fast in EDTA tubes. The blood was centrifuged at 1,900×g and 4°C for 15 min to obtain plasma, and then kept at -80°C until being analyzed. The plasma FGF23 concentrations were measured using an ELISA with a commercial reagent (Kainos Laboratories, Tokyo, Japan).[25] The concentration detection range was 3–800 pg/mL, and so the assay was repeated using a 1/10 dilution for concentrations >800 pg/mL.[25] The plasma FGF23 concentration was measured twice by two investigators blinded to the medical data (Y.C. and D.R.R.) and then averaged. Intra- and interassay coefficients of variability were 4.2% and 6.5%, respectively.

Statistical analysis

Statistical analyses were conducted using the SPSS software package for Windows (version 21.0, IBM Corp., Armonk, NY, USA). Continuous variables were analyzed using the independent *t*-test, Mann-Whitney test, one-way ANOVA with Bonferroni's post-hoc analysis, and Kruskal-Wallis test. Categorical variables were analyzed using the chi-square or Fisher's exact test. Univariate and multivariate binary logistic regression analyses were performed to investigate the association between the plasma FGF23 concentration and cerebral atherosclerosis. To investigate the associations of age, body mass index, stroke severity, time to blood sampling from symptom onset, and blood laboratory findings with FGF23, Spearman correlation analysis was performed for the correlations between the National Institutes of Health Stroke Scale (NIHSS) score, blood laboratory findings, and FGF23 concentration.

The association between the plasma FGF23 concentration and the burden of cerebral atherosclerosis was investigated by performing a Spearman correlation analysis and univariate and multivariate ordinal logistic regression analyses. Ordinal logistic regression was used to analyze the association between the FGF23 concentration and the number of arteries with cerebral atherosclerosis. The odds ratio (OR) in ordinal regression expresses the common odds for the increase in the number of arteries with cerebral atherosclerosis at each count. Sex, age, and variables for which $p<0.1$ in the univariate analysis were included in the multivariate logistic regression. In the multivariate analysis, sex, age, body mass index, coronary artery disease, prestroke antithrombotics, stroke subtype, NIHSS score, high-grade WMHs, Klotho, triglyceride, WBC count, total calcium, and C-reactive protein were adjusted for the presence and burden of ICAS; sex, age, hypertension, stroke subtype, NIHSS score, high-grade WMHs, WBC count, phosphate, and uric acid were adjusted for the presence and burden of ECAS; and sex, age, hypertension, prestroke antithrombotics, NIHSS score, high-grade WMHs, Klotho, triglyceride, WBC count, and C-reactive protein were adjusted for the presence and burden of both ICAS and ECAS. For sensitivity analysis, we investigated the presence of statistical interactions according to underlying renal dysfunction (<60 mL/min/1.73 m^2), which was closely related to the plasma FGF23 concentration.[11] A two-tailed probability value of $p<0.05$ was considered statistically significant.

RESULTS

Demographic data and comparative analysis according to presence of cerebral atherosclerosis

Fig. 1 shows a flowchart of participants according to the inclusion and exclusion criteria applied in this study. The demographics and accompanying risk factors and prior stroke medications did not differ significantly between the included and excluded patients (Supplementary Table 1 in the online-only Data Supplement). Table 1 lists the demographics of the patients in our study. The 262 patients included 58.0% (*n*=152) males. The mean age was 64.7 years, and the plasma FGF23 concentration was 347.5±549.6 pg/mL (mean±SD). The most common stroke subtype was large-artery atherosclerosis (41.2%, *n*=108), followed by small-vessel occlusion (40.8%, *n*=107) and cardioembolism (17.9%, *n*=47). The plasma FGF23 concentration did not differ among stroke subtypes ($p=0.174$). ICAS only, ECAS only, and both ICAS and ECAS were present in 31.2% (*n*=82), 4.9% (*n*=13), and 6.8% (*n*=18) of the subjects, respectively (Table 1). The patients with ICAS and both ICAS and ECAS were older ($p=0.002$)

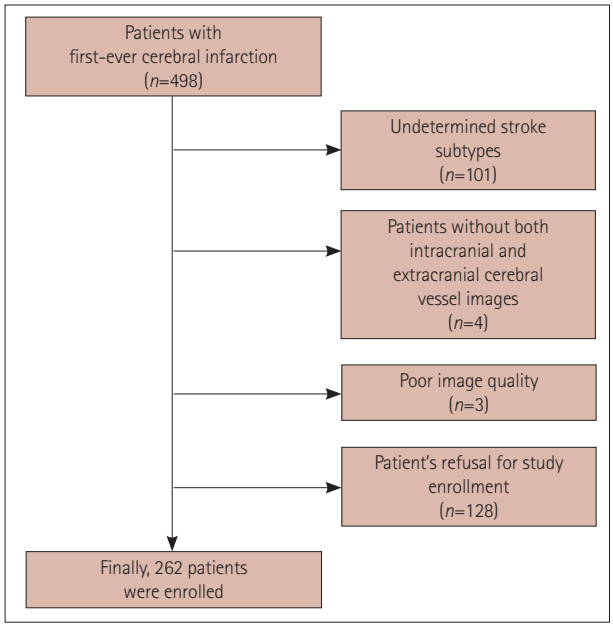

Fig. 1. Flowchart of participants according to inclusion and exclusion criteria.

and had higher NIHSS scores (p=0.002) than those without cerebral atherosclerosis. Moreover, patients with ICAS only, ECAS only, or both ICAS and ECAS more often had a history of hypertension (p=0.003) and large-artery atherosclerosis stroke subtype (p=0.001).

The blood laboratory findings indicated that the plasma FGF23 concentration (p=0.001), WBC count (p=0.014), and C-reactive protein concentration (p=0.017) were higher in patients with both ICAS and ECAS than in patients without cerebral atherosclerosis. Patients with ICAS had higher plasma concentrations of FGF23 (p=0.037) and C-reactive protein (p=0.043) compared with patients without cerebral atherosclerosis. Patients with both ICAS and ECAS had a higher plasma FGF23 concentration compared with patients with only

Table 1. Comparison of clinical characteristics and blood laboratory findings according the presence of cerebral atherosclerosis

	Total (n=262)	No cerebral atherosclerosis (n=149)	ECAS only (n=13)	ICAS only (n=82)	Both ECAS and ICAS (n=18)	p
Demographics						
Sex, male	152 (58.0)	89 (59.7)	10 (76.9)	43 (52.4)	10 (55.6)	0.365
Age, years	64.7±12.3	62.4±12.0	64.6±13.9	67.5±12.3*	71.5±8.5*	0.002
Body mass index, kg/m^2	24.0±3.3	23.7±2.8	23.0±2.2	24.6±4.1	24.3±3.9	0.186
Risk factors						
Hypertension	153 (58.4)	77 (51.7)	10 (76.9)	49 (59.8)	17 (94.4)	0.003
Diabetes mellitus	108 (41.2)	56 (37.6)	5 (38.5)	38 (46.3)	9 (50.0)	0.510
Hypercholesterolemia	74 (28.2)	40 (26.8)	2 (15.4)	27 (32.9)	5 (27.8)	0.553
Coronary artery disease	47 (17.9)	22 (14.8)	2 (15.4)	17 (20.7)	6 (33.3)	0.221
Smoking	98 (37.4)	56 (37.6)	5 (38.5)	31 (37.8)	6 (33.3)	0.986
Alcohol intake	73 (27.9)	48 (32.2)	4 (30.8)	17 (20.7)	4 (22.2)	0.282
Prior medication						
Antithrombotics	55 (21.0)	24 (16.1)	2 (15.4)	21 (25.6)	8 (44.4)	0.024
Statins	53 (20.2)	32 (21.5)	4 (30.8)	13 (15.9)	4 (22.2)	0.561
Stroke subtype						0.001
Small-vessel occlusion	107 (40.8)	85 (57.0)	2 (15.4)	16 (19.5)	4 (22.2)	
Large-artery atherosclerosis	108 (41.2)	37 (24.8)	9 (69.2)	51 (62.2)	11 (61.1)	
Cardioembolism	47 (17.9)	27 (18.1)	2 (15.4)	15 (18.3)	3 (16.7)	
NIHSS score	3 [2–6]	3 [2–5]	2 [1–8]	5 [2–8]*	6 [3–12]*	0.002
High-grade WMHs	64 (24.4)	30 (20.1)	4 (30.8)	22 (26.8)	8 (44.4)	0.115
Time to blood sampling from symptom onset, h	11.3 [4.6–20.4]	11.8 [5.0–21.1]	11.1 [4.1–19.8]	11.0 [4.2–19.6]	11.2 [4.4–20.6]	0.218
Blood laboratory findings						
FGF23, pg/mL	347.5±549.6	255.9±348.3	153.0±71.7	394.4±617.4*	1033.0±1073.1*†‡	0.001
Klotho, pg/mL	312.7±153.3	327.0±140.4	382.1±253.4	285.1±142.2	270.1±190.8	0.045
Vitamin D [25(OH)D], ng/mL	20.1±6.8	19.9±7.0	17.9±6.6	21.1±6.7	19.7±5.4	0.350
HbA1c, %	6.5±1.4	6.4±1.3	6.9±1.7	6.7±1.5	6.4±1.1	0.224
Triglyceride, mg/dL	129.0±95.6	121.8±90.3	101.3±37.2	141.7±114.0	150.6±63.6	0.233
Low-density lipoprotein, mg/dL	115.0±36.9	112.2±35.4	111.6±49.7	118.5±37.9	124.6±33.9	0.401
White blood cell count, ×10^3	7.3±2.4	7.1±2.1	7.9±3.7	7.4±2.4	9.0±2.7*	0.014
Creatinine, mg/dL	1.0±0.8	1.0±0.9	0.9±0.1	0.9±0.3	1.3±1.5	0.182
Total calcium, mg/dL	8.2±0.4	8.2±0.4	8.2±0.4	8.3±0.4	8.3±0.6	0.375
Phosphate, mg/dL	3.1±0.6	3.1±0.5	3.3±0.6	3.1±0.5	3.5±1.0	0.083
Alkaline phosphatase, IU/L	224.4±72.2	225.1±70.4	209.0±45.1	227.8±81.6	213.7±58.0	0.761
Uric acid, mg/dL	4.8±1.6	4.8±1.5	5.5±1.9	4.6±1.5	5.2±2.1	0.288
CRP, mg/L	0.9±2.8	0.5±1.3	0.7±1.1	1.5±1.3*	2.2±3.2*	0.017

Data are n (%), mean±SD, or median [interquartile range] values.
*p<0.05 compared with no cerebral atherosclerosis in a Bonferroni post-hoc analysis, †p<0.05 compared with ECAS only in a Bonferroni post-hoc analysis, ‡p<0.05 compared with ICAS only in a Bonferroni post-hoc analysis.
ECAS: extracranial cerebral atherosclerosis, FGF23: fibroblast growth factor 23, ICAS: intracranial cerebral atherosclerosis, NIHSS: National Institute of Health Stroke Scale, WMHs: white-matter hyperintensities.

ICAS (p=0.001) or only ECAS (p=0.001) (Table 1).

The plasma FGF23 concentration was positively correlated with age (ρ=0.150, p=0.015), NIHSS score (ρ=0.198, p=0.001), vitamin D [25(OH)D] concentration (ρ=0.143, p=0.020), HbA1c (ρ=0.162, p=0.008), triglyceride concentration (ρ=0.319, p<0.001), WBC count (ρ=0.130, p=0.035), and creatinine (ρ=0.178, p=0.004), total calcium (ρ=0.153, p=0.043), phosphate (ρ=0.148, p=0.048), and C-reactive protein (ρ=0.134, p=0.030) concentrations. Moreover, the plasma FGF23 concentration was negatively correlated with the Klotho concentration (ρ=-0.325, p<0.001).

Association of FGF23 with presence of cerebral atherosclerosis

In a multivariate binary logistic analysis, the plasma FGF23 concentration was positively correlated with the presence of ICAS even after adjustment as a continuous variable (per 100 pg/mL) (OR=1.07, 95% CI=1.00−1.15, p=0.039) or dichotomizing based on the median value (cutoff value=182.0 pg/mL; OR=2.52, 95% CI=1.36−4.68, p=0.003) and tertiles [comparing the upper tertile (≥235.16 pg/mL) with the lower tertile (0−146.79 pg/mL): OR=3.28, 95% CI=1.53−7.05] (Table 2, Supplementary Table 2 in the online-only Data Supplement). The plasma FGF23 concentration was marginally related to the presence of ECAS after adjustment as a continuous variable (per 100 pg/mL; OR=1.05, 95% CI=0.99−1.12, p=0.073), but it was not associated with the presence of ECAS after adjustment using the median cutoff value or tertiles (Table 2, Supplementary Table 2 in the online-only Data Supplement). The plasma FGF23 concentration was significantly and positively correlated with the presence of both ICAS and ECAS after adjustment as a continuous variable (per 100 pg/mL; OR=1.10, 95% CI=1.02−1.19, p=0.013) (Table 2, Supplementary Table 2 in the online-only Data Supplement).

There was no statistical interaction between the presence of ICAS (p for interaction=0.872) and ECAS (p for interaction=0.764) with the presence of renal dysfunction (<60 mL/min/1.73 m^2).

Association of FGF23 with the burden of cerebral atherosclerosis

The plasma FGF23 concentration was associated with the ICAS burden (ρ=0.317, p=0.001) and the ECAS burden (ρ=0.145, p=0.019). The association of FGF23 with the burden of cerebral atherosclerosis is presented as Fig. 2. In a multivariate ordinal logistic analysis, the plasma FGF23 concentration was positively correlated with the burden of ICAS even after adjustment as a continuous variable (per 100 pg/mL; OR=1.09, 95% CI=1.04−1.15, p=0.001) or dichotomizing based on the median value (cutoff value=182.0 pg/mL; OR=1.10, 95% CI=1.58−5.06, p=0.001) and tertiles [comparing the upper tertile (≥235.16 pg/mL) with the lower tertile (0−146.79 pg/mL): OR=3.30, 95% CI=1.60−6.75] (Table 2, Supplementary Table 3 in the online-only Data Supplement). The plasma FGF23 concentration was related to the burden of ECAS after adjustment as a continuous variable (per 100 pg/mL; OR=1.06, 95% CI=1.00−1.12, p=0.038) but not after adjustment using the median cutoff value or tertiles (Table 2, Supplementary Table 3 in the online-only Data Supplement). The plasma FGF23 concentration was significantly and positively correlated with the burden of both ICAS and ECAS after adjustment as a continuous variable (per 100 pg/mL; OR=1.10, 95% CI=1.02−1.19, p=0.013) (Table 2, Supplementary Table 3 in the online-only Data Supplement).

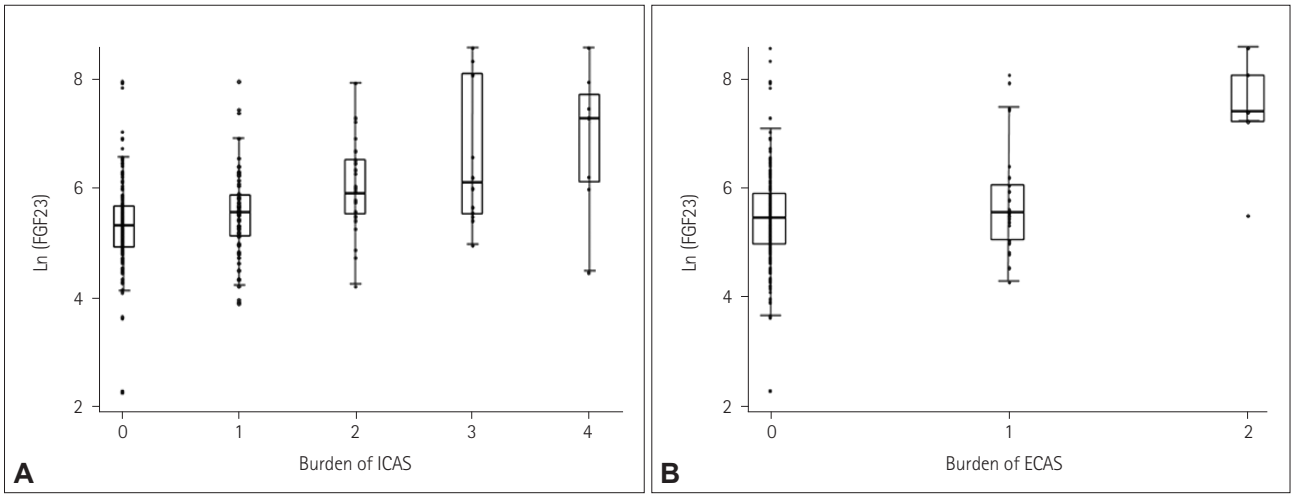

Fig. 2. Association of FGF23 with the burden of ICAS (A) and the burden of ECAS (B). X-axis indicates the burden of each type of cerebral atherosclerosis, and Y-axis indicates the value of natural logarithm of FGF23. ECAS: extracranial cerebral atherosclerosis, FGF23: fibroblast growth factor 23, ICAS: intracranial cerebral atherosclerosis.

Table 2. Results of multivariate analyses of the presence and burden of cerebral atherosclerosis

FGF23	Presence of cerebral atherosclerosis					
	ICAS†	*p*	ECAS†	*p*	Both ECAS and ICAS§	*p*
Continuous variable, per 100 pg/mL	1.07 (1.00–1.15)	0.039	1.05 (0.99–1.12)	0.073	1.10 (1.02–1.19)	0.013
Dichotomized based on median value of 182.0 pg/mL	2.52 (1.36–4.68)	0.003	1.59 (0.66–3.87)	0.248	Not available*	
Tertiles						
Lower tertile, 0–146.79 pg/mL	Reference		Reference		Reference	
Middle tertile, 146.79–235.16 pg/mL	1.96 (0.93–4.12)	0.076	1.97 (0.64–6.08)	0.482	Not available*	
Upper tertile, ≥235.16 pg/mL	3.28 (1.53–7.05)	0.002	1.26 (0.42–3.75)	0.523	Not available*	
FGF23	**Burden of cerebral atherosclerosis**					
	ICAS†	*p*	ECAS†	*p*	Both ECAS and ICAS§	*p*
Continuous variable, per 100 pg/mL	1.09 (1.04–1.15)	0.001	1.06 (1.00–1.12)	0.038	1.10 (1.02–1.19)	0.013
Dichotomized based on median value of 182.0 pg/mL	1.10 (1.58–5.06)	0.001	1.63 (0.66–3.99)	0.283	Not available*	
Tertiles						
Lower tertile, 0–146.79 pg/mL	Reference		Reference		Reference	
Middle tertile, 146.79–235.16 pg/mL	2.27 (1.10–4.67)	0.001	1.35 (0.44–4.11)	0.590	Not available*	
Upper tertile, ≥235.16 pg/mL	3.30 (1.60–6.75)	0.026	2.09 (0.66–6.53)	0.204	Not available*	

Data are odds ratio (95% CI) values.
*Odds ratio could not be obtained because the FGF23 concentrations of patients with both ECAS and ICAS exceeded 182.0 pg/mL, †Adjusted for sex, age, BMI, coronary artery disease, prestroke antithrombotics, stroke subtype, NIHSS score, high-grade WMHs, Klotho, triglyceride, WBC count, total calcium, and CRP, †Adjusted for sex, age, hypertension, stroke subtype, NIHSS score, high-grade WMHs, WBC count, phosphate, and uric acid, §Adjusted for sex, age, hypertension, prestroke antithrombotics, NIHSS score, high-grade WMHs, Klotho, triglyceride, WBC count, and CRP.
BMI: body mass index, ECAS: extracranial cerebral atherosclerosis, FGF23: fibroblast growth factor 23, ICAS: intracranial cerebral atherosclerosis, NIHSS: National Institutes of Health Stroke Scale, WBC: white blood cell, WMHs: white-matter hyperintensities.

DISCUSSION

The key finding of our study is that a higher plasma FGF23 concentration was associated with the presence and burden of cerebral atherosclerosis, particularly for ICAS. An association of FGF23 with cerebral atherosclerosis has rarely been reported. A community-based study found that a higher circulating FGF23 concentration was associated with systemic atherosclerosis.[26] Moreover, a higher FGF23 concentration was found to be a risk factor for chronic kidney diseases, especially in older, disabled, community-dwelling females.[27] In patients with chronic kidney diseases, an elevated FGF23 concentration reportedly contributes directly to a higher rate of left ventricular hypertrophy[28] and a higher coronary artery disease burden.[29] Combined with previous research, the present study is significant because it confirms the association between the plasma FGF23 concentration and the presence and burden of cerebral atherosclerosis, specifically in stroke patients.

While our study cannot suggest the exact mechanism linking plasma FGF23 and cerebral atherosclerosis, there are plausible hypotheses. First, vascular inflammation of cerebral arteries may explain the relationship between FGF23 and cerebral atherosclerosis. Previous studies have found FGF23 to be an important mediator of vessel inflammation that preceded arteriolosclerosis or arterial stiffness, which was the main cause of cerebral atherosclerosis development, particularly for ICAS.[22,30] Moreover, FGF23 stimulates the hepatic secretion of the inflammatory markers IL-6 and C-reactive protein.[30] C-reactive protein is a sensitive indicator of vascular inflammation and a marker of cerebral atherosclerosis.[31] In line with these previous results, the present study found that the plasma FGF23 concentration was positively correlated with that of C-reactive protein, with the latter also being higher in patients with accompanying ICAS than in those without cerebral atherosclerosis. Second, FGF23 is a phosphaturic hormone produced mainly by osteoblasts and osteocytes, and is implicated in blood calcium and phosphate concentrations, the vitamin D pathway, and ectopic site mineralization.[5] Increased FGF23 activity disturbs the calcitriol-calcium/phosphate regulation pathway, which may cause hypercalcemia and hyperphosphatemia, likely accounting for the association with ectopic site mineralization.[32,33] Previous studies have demonstrated the serum calcium concentration to be positively correlated with the presence of ICAS; in contrast, the serum phosphate concentration was not associated with ICAS.[34] Furthermore, the phosphate concentration was positively correlated with a greater prevalence of vascular calcification, which is frequently associated with ECAS.[35,36] Therefore, an FGF23-related calcium-phosphate regulation mechanism may affect the development of cerebral atherosclerosis. Actually, the present study found that the plasma FGF23 was positively correlated with serum calcium and phosphate concentrations, and also that the serum calcium and phosphate

concentrations were related to the presence and burden of ICAS and ECAS, respectively.

This study found that the presence and burden of ICAS were significantly associated with a higher FGF23 concentration, whereas the presence and burden of ECAS were not, even though they were related in a univariate analysis. These results may have been due to the sample being too small to reveal a relationship between FGF23 and ECAS. Moreover, other confounding factors such as hypertension are more strongly associated with ECAS than with the FGF23 concentration. In addition, arterial stiffness may be associated with ICAS (rather than ECAS) in Asians,[22] and arterial stiffness is also closely related to FGF23,[37] which may explain our results. Our study also suggests the presence of a pathophysiological association between circulating FGF23 and ICAS.

This study was subject to some limitations. We did not measure the plasma FGF23 concentration in the general population. However, the main goals of our study were to determine any associations with the presence and burden of cerebral atherosclerosis in stroke patients. All blood samples and brain imaging findings in this study were acquired from acute stroke patients at the time of admission, and so serial changes in the FGF23 concentration and the association of FGF23 with the long-term progression of cerebral atherosclerosis could not be investigated. Although our study prospectively enrolled ischemic stroke patients, it is difficult to generalize our findings and selection bias might have been present due to the smallness of the sample and the exclusion of undetermined stroke subtypes. Even though the plasma FGF23 concentration was found to be associated with the presence of ICAS and ECAS, it did not differ among stroke subtypes. This may have been due to the cardioembolic stroke mechanism or the sample smallness reducing the statistical power. Finally, we did not measure thyroid and parathyroid hormones that may affect the FGF23 concentration, or examine bone abnormalities in the enrolled patients.

In conclusion, this study has demonstrated that a higher plasma FGF23 concentration is independently associated with the presence and burden of cerebral atherosclerosis, particularly with ICAS in stroke patients. We attribute these associations to the essential role of FGF23 in cerebral atherosclerosis.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.3988/jcn.2020.16.1.29.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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REFERENCES

1. Bang OY. Intracranial atherosclerosis: current understanding and perspectives. *J Stroke* 2014;16:27-35.
2. Arenillas JF, Molina CA, Chacón P, Rovira A, Montaner J, Coscojuela P, et al. High lipoprotein (a), diabetes, and the extent of symptomatic intracranial atherosclerosis. *Neurology* 2004;63:27-32.
3. Park KY, Chung CS, Lee KH, Kim GM, Kim YB, Oh K. Prevalence and risk factors of intracranial atherosclerosis in an asymptomatic Korean population. *J Clin Neurol* 2006;2:29-33.
4. Song TJ, Park JH, Choi KH, Kim JH, Choi Y, Chang Y, et al. Is obstructive sleep apnea associated with the presence of intracranial cerebral atherosclerosis? *Sleep Breath* 2017;21:639-646.
5. ADHR Consortium. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* 2000;26:345-348.
6. Martin A, David V, Quarles LD. Regulation and function of the FGF23/Klotho endocrine pathways. *Physiol Rev* 2012;92:131-155.
7. Zoccali C, Yilmaz MI, Mallamaci F. FGF23: a mature renal and cardiovascular risk factor? *Blood Purif* 2013;36:52-57.
8. Donate-Correa J, Muros de Fuentes M, Mora-Fernández C, Navarro-González JF. Pathophysiological implications of fibroblast growth factor-23 and Klotho and their potential role as clinical biomarkers. *Clin Chem* 2014;60:933-940.
9. Hu X, Ma X, Luo Y, Xu Y, Xiong Q, Pan X, et al. Elevation in fibroblast growth factor 23 and its value for identifying subclinical atherosclerosis in first-degree relatives of patients with diabetes. *Sci Rep* 2016;6:34696.
10. Shibata K, Fujita S, Morita H, Okamoto Y, Sohmiya K, Hoshiga M, et al. Association between circulating fibroblast growth factor 23, α-Klotho, and the left ventricular ejection fraction and left ventricular mass in cardiology inpatients. *PLoS One* 2013;8:e73184.
11. Malyszko J, Koc-Zorawska E, Matuszkiewicz-Rowinska J, Malyszko J. FGF23 and Klotho in relation to markers of endothelial dysfunction in kidney transplant recipients. *Transplant Proc* 2014;46:2647-2650.
12. Ärnlöv J, Carlsson AC, Sundström J, Ingelsson E, Larsson A, Lind L, et al. Serum FGF23 and risk of cardiovascular events in relation to mineral metabolism and cardiovascular pathology. *Clin J Am Soc Nephrol*

2013;8:781-786.
13. Schoppet M, Hofbauer LC, Brinskelle-Schmal N, Varennes A, Goudable J, Richard M, et al. Serum level of the phosphaturic factor FGF23 is associated with abdominal aortic calcification in men: the STRAMBO study. *J Clin Endocrinol Metab* 2012;97:E575-E583.
14. Song TJ, Kim YD, Yoo J, Kim J, Chang HJ, Hong GR, et al. Association between aortic atheroma and cerebral small vessel disease in patients with ischemic stroke. *J Stroke* 2016;18:312-320.
15. Chang Y, Kim J, Kim MH, Kim YJ, Song TJ. Interarm blood pressure difference is associated with early neurological deterioration, poor short-term functional outcome, and mortality in noncardioembolic stroke patients. *J Clin Neurol* 2018;14:555-565.
16. Song TJ, Chang Y, Chun MY, Lee CY, Kim AR, Kim Y, et al. High dietary glycemic load is associated with poor functional outcome in patients with acute cerebral infarction. *J Clin Neurol* 2018;14:165-173.
17. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24:35-41.
18. Song TJ, Kim J, Yang SH, Park JH, Lee HS, Nam CM, et al. Association of plasma osteoprotegerin levels with stroke severity and functional outcome in acute ischaemic stroke patients. *Biomarkers* 2012;17:738-744.
19. Song TJ, Kim J, Kim YD, Nam HS, Lee HS, Nam CM, et al. The distribution of cerebral microbleeds determines their association with arterial stiffness in non-cardioembolic acute stroke patients. *Eur J Neurol* 2014;21:463-469.
20. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;145:247-254.
21. Samuels OB, Joseph GJ, Lynn MJ, Smith HA, Chimowitz MI. A standardized method for measuring intracranial arterial stenosis. *AJNR Am J Neuroradiol* 2000;21:643-646.
22. Kim J, Cha MJ, Lee DH, Lee HS, Nam CM, Nam HS, et al. The association between cerebral atherosclerosis and arterial stiffness in acute ischemic stroke. *Atherosclerosis* 2011;219:887-891.
23. North American Symptomatic Carotid Endarterectomy Trial Collaborators, Barnett HJM, Taylor DW, Haynes RB, Sackett DL, Peerless SJ, et al. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med* 1991;325:445-453.
24. Chang Y, Choi GS, Lim SM, Kim YJ, Song TJ. Interarm systolic and diastolic blood pressure difference is diversely associated with cerebral atherosclerosis in noncardioembolic stroke patients. *Am J Hypertens* 2017;31:35-42.
25. Lima F, El-Husseini A, Monier-Faugere MC, David V, Mawad H, Quarles D, et al. FGF-23 serum levels and bone histomorphometric results in adult patients with chronic kidney disease on dialysis. *Clin Nephrol* 2014;82:287-295.
26. Mirza MA, Hansen T, Johansson L, Ahlström H, Larsson A, Lind L, et al. Relationship between circulating FGF23 and total body atherosclerosis in the community. *Nephrol Dial Transplant* 2009;24:3125-3131.
27. Semba RD, Fink JC, Sun K, Cappola AR, Dalal M, Crasto C, et al. Serum fibroblast growth factor-23 and risk of incident chronic kidney disease in older community-dwelling women. *Clin J Am Soc Nephrol* 2012;7:85-91.
28. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011;121:4393-4408.
29. Kanbay M, Nicoleta M, Selcoki Y, Ikizek M, Aydin M, Eryonucu B, et al. Fibroblast growth factor 23 and fetuin A are independent predictors for the coronary artery disease extent in mild chronic kidney disease. *Clin J Am Soc Nephrol* 2010;5:1780-1786.
30. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, et al. Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. *Kidney Int* 2016;90:985-996.
31. Xie D, Deng L, Liu XD, Li JM, Zhang YB. Role of high sensitivity C-reactive protein and other risk factors in intracranial and extracranial artery occlusion in patients with ischaemic stroke. *J Int Med Res* 2015;43:711-717.
32. Lanske B, Razzaque MS. Molecular interactions of FGF23 and PTH in phosphate regulation. *Kidney Int* 2014;86:1072-1074.
33. Rodriguez-Ortiz ME, Lopez I, Muñoz-Castañeda JR, Martinez-Moreno JM, Ramírez AP, Pineda C, et al. Calcium deficiency reduces circulating levels of FGF23. *J Am Soc Nephrol* 2012;23:1190-1197.
34. Kang K. Serum calcium and phosphate concentrations and intracranial atherosclerosis. *Atherosclerosis* 2014;232:249-253.
35. Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, et al. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol* 2009;20:381-387.
36. Pini R, Faggioli G, Fittipaldi S, Vasuri F, Longhi M, Gallitto E, et al. Relationship between calcification and vulnerability of the carotid plaques. *Ann Vasc Surg* 2017;44:336-342.
37. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis* 2009;205:385-390.