Serum Sortilin Is Associated with Coronary Artery Calcification and Cardiovascular and Cerebrovascular Events in Maintenance Hemodialysis Patients

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Abstract

Objective: To analyze the role of serum sortilin in coronary artery calcification (CAC) and cardiovascular and cerebrovascular events (CCE) in maintenance hemodialysis (MHD) patients. Methods: One hundred eleven patients with MHD ≥3 months were included in this study. The general data, clinical features, hematological data, and medication history of the patients were recorded. Eighty-five cases were examined by vascular color Doppler ultrasound, cardiac color Doppler ultrasound, lateral lumbar radiography, and coronary artery calcification score. The patients were followed up for a median time of 45 months. The primary endpoint was CCE or death from a vascular event, and the role of sortilin in this process was analyzed. Results: Among 85 MHD patients, 51 cases (60.00%) had different degrees of CAC. There were significant differences in diabetes, dialysis time, serum phosphorus, calcium-phosphorus product, medical history of phosphate binders, sortilin, and carotid artery plaque between 4 different degrees of calcification groups (p < 0.05). Logistic regression analysis showed that diabetes (OR = 5.475; 95% CI: 1.794–16.71, p = 0.003), calcium-phosphorus product (OR = 2.953; 95% CI: 1.198–7.279, p = 0.019), and sortilin (OR = 1.475 per 100 pg/mL; 95% CI: 1.170–1.858, p = 0.001) were independent risk factors for CAC. During the follow-up, 28 cases of 111 patients (25.23%) suffered from CCE. There were significant differences in CCE between mild, moderate, and severe CAC groups and noncalcification.

Keywords

Maintenance hemodialysis · Sortilin · Coronary artery calcification · Cardiovascular and cerebrovascular events

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groups ($p < 0.05$). Cox regression analysis showed that diabetes mellitus (HR 3.424; 95% CI: 1.348–8.701, $p = 0.010$), CAC (HR 5.210; 95% CI: 1.093–24.83, $p = 0.038$), and serum sortilin (HR = 8.588; 95% CI: 1.919–38.43, $p = 0.005$) were independent risk factors for CCE. Besides, we proposed a cutoff value of 418 pg/mL for serum sortilin level, which was able to predict the occurrence of CCE with 75.0% sensitivity and 71.9% specificity. The area under the curve was 0.778 (95% CI: 0.673–0.883).

Conclusion: Sortilin is newly found to be independently associated with CAC and CCE in MHD patients.

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Materials and Methods

Study Subjects
This prospective study was conducted from January 2015 to September 2018. A total of 111 adult patients (21–75 years old) who had regular MHD for >3 months in the blood purification center of Xiangya Hospital of Central South University from January to March 2015 were enrolled. The expected survival time was >6 months. Patients were not eligible for study inclusion if any of the following were present: acute infection and inflammatory disease within 1 month; malignant tumor; pregnancy; mental disease; previous CCE, including angina pectoris, myocardial infarction, heart failure, arrhythmia, transient ischemia attack, and stroke; and previous renal transplantation or parathyroid gland resection. The dialysis regimen was 3 times/week or 5 times/2 weeks, 4 h/ time, calcium concentration of the dialysate was 1.5 mmol/L, blood flow was 200–280 mL/min, and the dialysate flow rate was 500 mL/min. The goal of ultrafiltration was to achieve the clinical estimated dry weight.

Baseline Data Collection
All data were abstracted retrospectively from electronic medical records and included demographic characteristics, comorbidities, laboratory findings, therapies, and outcomes. The body weight and blood pressure were measured on the morning of dialysis. The blood routine, blood lipid, high-sensitivity C-reactive protein (CRP), calcium, phosphorus, and serum iPTH were detected in the morning after dialysis. After centrifugation, 5 mL blood was separated and stored at −80°C. All samples were thawed only once before use. Circulating serum sortilin was measured using a commercial ELISA kit (Cusabio ELISA kit; Cosmo Bio, Carlsbad, CA, USA). The ELISA kit can detect sortilin levels ranged from 46.88 to 3,000 pg/mL, and the coefficient of variation of internal precision is <8%.

Imaging Examination
Multislice spiral computed tomography was performed on the same day or the next day of dialysis. The CAC score (CACs) was evaluated by a trained radiologist according to the blind method and using German Siemens CaScoring software. The CACs was the calcified plaque area multiplied by the fixed coefficient (determined by the maximum pixel density). Calcified plaques were defined as lesions with CT value ≥130 HU and area ≥1 mm² [12]. The CACs was divided into 4 groups according to the Rumberger classification of CAC [13]. CACs ≤10 was defined as non-CAC, 11–100 was defined as mild CAC, 101–400 was defined as moderate CAC, and >400 was defined as severe CAC. The lateral lumbar
radiography was taken to evaluate abdominal aortic calcification. Echocardiography showed cardiac valvular calcification (ValvC), defined as bright echoes of >1 mm thickness are seen on one or more cusps of the aortic valve, mitral valve, or mitral annulus [14]. Baseline echocardiography was evaluated using the left ventricular mass index (LVMI). Left ventricular hypertrophy was scored if LVMI was >125 g/m\(^2\) in males or >120 g/m\(^2\) in females. Carotid plaque was measured by color Doppler ultrasound. The plaque was defined as a localized echo structure protruding from the lumen (echo may be uneven or accompanied by acoustic shadow), thickness ≥1.3 mm. B-ultrasound examination and reading were completed by the same ultrasound doctor in our hospital.

**Follow-Up**

Blood calcium, phosphorus, and iPTH were detected regularly in all patients every half a year, and drugs were adjusted according to the test results. The occurrence of CCE and survival were recorded until the 45th month or the patients’ death. The primary endpoint was defined as CCE or death from a vascular event. CCE include angina pectoris, myocardial infarction, coronary revascularization, congestive heart failure, transient ischemic attack (TIA), and stroke [15]. Angina pectoris was defined using NICE clinical guideline concerning chest pain of recent onset [16]. Myocardial infarction was defined following ESC/ACCF/AHA/WHF Expert Consensus Document (2007) [17]. Congestive heart failure is char-

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**Table 1. Clinical and biochemical characteristics of MHD patients with different degrees of calcification**

| Parameter                      | Non-CAC (n = 34) | Mild CAC (n = 16) | Moderate CAC (n = 20) | Severe CAC (n = 15) | p value |
|--------------------------------|------------------|-------------------|-----------------------|---------------------|---------|
| Age, years                     | 44±12            | 52±14             | 49±8                  | 52±12               | 0.063   |
| Male, n (%)                    | 12 (35.29)       | 9 (56.25)         | 13 (65.00)            | 10 (66.67)          | 0.087   |
| BMI, kg/m\(^2\)                | 21.65±2.86       | 22.71±2.48        | 21.90±2.31            | 22.03±3.19          | 0.466   |
| Current smokers, n (%)         | 6 (17.65)        | 7 (43.75)         | 9 (45.00)             | 7 (46.67)           | 0.076   |
| Hypertension, n (%)            | 26 (76.47)       | 15 (93.75)        | 19 (95.00)            | 14 (93.33)          | 0.129   |
| DM, n (%)                      | 3 (8.82)         | 3 (18.75)         | 6 (30.00)             | 7 (46.67)           | **0.024** |
| SBP, mm Hg                     | 142±19           | 143±19            | 154±20                | 151±19              | 0.088   |
| DBP, mm Hg                     | 85±13            | 83±10             | 92±11                 | 92±13               | 0.063   |
| Dialysis, months               | 25 (17, 36)      | 24 (6, 59)        | 36 (24, 54)           | 47 (40, 90)         | **0.012** |
| Medication history, n (%)      |                  |                   |                       |                     |         |
| CCB                            | 23 (67.65)       | 12 (75.00)        | 18 (90.00)            | 13 (86.67)          | 0.214   |
| ACEI/ARB                       | 9 (26.47)        | 6 (37.50)         | 8 (40.00)             | 2 (13.33)           | 0.309   |
| α-β-Blocker/α-blocker          | 21 (61.76)       | 8 (50.00)         | 13 (65.00)            | 11 (73.77)          | 0.599   |
| β-Blocker                      | 13 (38.24)       | 8 (50.00)         | 5 (25.00)             | 7 (46.67)           | 0.416   |
| Calcium supplement             | 13 (38.24)       | 9 (56.25)         | 6 (30.00)             | 5 (33.33)           | 0.408   |
| Vitamin D analogs              | 23 (67.65)       | 11 (68.75)        | 15 (75.00)            | 14 (93.33)          | 0.275   |
| Phosphate binders              | 8 (23.53)        | 3 (18.75)         | 12 (60.00)            | 5 (33.33)           | **0.024** |
| Hb, g/L                        | 96±16            | 96±15             | 95±17                 | 100±13              | 0.867   |
| PLT, ×10\(^9\)/L               | 154±55           | 165±57            | 149±34                | 146±38              | 0.708   |
| TG, mmol/L                     | 1.14 (0.83, 1.87) | 1.05 (0.58, 1.59) | 1.63 (1.08, 2.38)     | 0.93 (0.62, 1.90)   | 0.092   |
| TC, mmol/L                     | 4.11±1.28        | 3.67±1.09         | 4.41±1.16             | 4.09±1.00           | 0.325   |
| HDL-C, mmol/L                  | 1.27±0.45        | 1.20±0.31         | 1.19±0.39             | 1.18±0.29           | 0.821   |
| LDL-C, mmol/L                  | 2.26±0.83        | 2.27±0.95         | 2.67±0.94             | 2.36±0.82           | 0.389   |
| Ca, mmol/L                     | 2.17±0.20        | 2.24±0.17         | 2.28±0.28             | 2.31±0.22           | 0.159   |
| P, mmol/L                      | 1.87±0.47        | 1.99±0.46         | 2.19±0.59             | 2.22±0.37           | **0.049** |
| Ca × P, mmol/L\(^2\)           | 4.06±3.00        | 4.46±1.09         | 4.98±1.39             | 5.17±1.25           | **0.007** |
| PTH, pg/mL                      | 319 (114, 405)   | 425 (121, 742)    | 354 (123, 566)        | 451 (247, 825)      | 0.268   |
| hs-CRP, mg/L                   | 0.89 (0.43, 2.65) | 1.92 (0.35, 8.51) | 2.99 (0.53, 9.98)     | 1.70 (0.20, 7.78)   | 0.677   |
| LVMI, g/m\(^2\)                | 128±46           | 134±28            | 145±37                | 138±31              | 0.336   |
| LVH, n (%)                     | 17 (50.00)       | 11 (68.75)        | 17 (85.00)            | 11 (73.33)          | 0.073   |
| Carotid plaque, n (%)           | 11 (32.35)       | 10 (62.50)        | 15 (75.00)            | 12 (80.00)          | **0.019** |
| ValvC, n (%)                    | 7 (20.59)        | 4 (25.00)         | 4 (20.00)             | 9 (60.00)           | 0.346   |
| AAC, n (%)                      | 9 (26.47)        | 9 (56.25)         | 9 (45.00)             | 10 (66.67)          | 0.148   |

Continuous variables are shown as mean±standard deviation or median and interquartile range. Categorical variables are shown as counts and percentages. p values for one-way ANOVA test or Kruskal-Wallis H test or χ\(^2\) test. Bold represents p < 0.05. MHD, maintenance hemodialysis; CAC, coronary artery calcification; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; Hb, hemoglobin; PLT, platelet; TG, triglyceride; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; PTH, parathyroid hormone; LVMI (left ventricular mass index) = LVM (left ventricular mass)/BSA (body surface area); LVH, left ventricular hypertrophy; ValvC, valve calcification; AAC, abdominal aortic calcification.
Serum sortilin levels in MHD patients with different degrees of CAC. Sortilin levels in serum were compared among 4 groups: MHD with non-CAC (n = 34), MHD with mild CAC (n = 16), MHD with moderate CAC (n = 20), and MHD with severe CAC (n = 15). Statistical significance was determined by the Kruskal-Wallis H test (***p < 0.001). MHD, maintenance hemodialysis; CAC, coronary artery calcification.

Baseline Clinical Characteristics
A total of 111 MHD patients with an average age of 49 ± 12 years were included in this study, of whom 60 (54.05%) were male. In this study, various etiologies of MHD reported in our center included chronic glomerulonephritis (58.6%), diabetes (17.2%), hypertension (10.8%), obstruction (7.2%), and others (6.3%). Among 111 MHD patients, 85 cases were examined by vascular color Doppler ultrasound, cardiac color Doppler ultrasound, lateral abdominal X-ray, and CACs. The total calcification rate of these 85 patients in this center was 76.6%, the CAC rate was 60.0%, the AAC rate was 36.5%, and the heart ValvC rate was 22.4%, which were slightly lower than the results of the dialysis calcification study (CDCS) published in 24 Chinese mainland centers in 2018 [20] perhaps because of the exclusion of the patients who had suffered CCE or parathyroid gland resection before enrollment. The range of CACs in 85 patients ranged from 0 to 3,071, with a median of 48.3. According to the Rumberger classification of CAC, there were 34 cases (40.00%) in the noncalcification group, 16 cases (18.82%) in the mild calcification group, 20 cases (23.53%) in the moderate calcification group, and 15 cases (17.65%) in the severe calcification group.

The clinical and biochemical characteristics of hemodialysis patients with different degrees of calcification are listed in Table 1. It could be seen that dialysis time, serum phosphorus, and calcium-phosphorus product levels were higher in moderate and severe CAC than their counterparts in non-CAC groups. More subjects had diabetes mellitus or carotid plaque with the aggravation of CAC. Besides, the medical history of phosphate binders was also related to the degree of calcification.

Serum Sortilin Levels and CAC
The serum levels of sortilin in noncalcification, mild calcification, moderate, and severe coronary calcification groups were 339.1 ± 144.6, 400.1 ± 137.4, 520.5 ± 214.2, and 648.9 ± 337.2 pg/mL, respectively (Fig. 1). Next, we transformed sortilin into sortilin/100 and included it in the logistic regression equation with diabetes history, dialysis time, blood phosphorus, calcium-phosphorus product, medicine history of phosphate binders, and carotid plaque. The results showed that diabetes, calcium-phosphorus product, and sortilin are independently related to CAC (Fig. 2). For every 100 pg/mL increase of sortilin, the risk of CAC increased by 1.475 times. The risk of CAC increased by 2.953 times...
with the increase of 1 (mmol/L)² calcium-phosphorus product. Besides, CAC risk in patients with diabetes was 5.475 times higher than that in patients without diabetes.

**Serum Sortilin Levels and CCE**

During the follow-up period of 45 months, 28 patients suffered from CCE. Fifteen MHD patients died during the period of follow-up: 8 patients died from vascular disorder, 5 died from infection, 1 died from liver cancer, and 1 died from severe malnutrition. The number of patients who suffered from angina pectoris, myocardial infarction, congestive heart failure, transient ischemic attack, and stroke was 12, 3, 18, 2, and 2, respectively (Fig. 3). Then, we compared the variables in subjects with or without CCE (Table 2). The subjects who had suffered from CCE had longer dialysis time and higher calcium-phosphorus product \( (p < 0.05) \). Diabetes mellitus, carotid plaque, and AAC all contributed to the occurrence of CCE in this study \( (p < 0.05) \). Besides, the serum sortilin level in patients with CCE was

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**Table 2**: OR and p value of CCE variables

| Variable          | OR (95% CI)     | p value |
|-------------------|-----------------|---------|
| Dialysis          | 1.017 (1.000–1.035) | 0.056   |
| DM                | 0.135 (0.016–1.122) | 0.064   |
| Phosphate binder  | 0.851 (0.312–2.317) | 0.752   |
| Carotid plaque    | 1.475 (1.170–1.858) | 0.001   |
| Ca*P              | 1.643 (0.622–4.342) | 0.316   |
| Sortilin per 100 pg/mL | 2.953 (1.198–7.279) | 0.031   |
| DM                | 5.475 (1.794–16.714) | 0.003   |

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**Fig. 2.** Forest map of CAC multivariate logistic regression analysis. Sortilin was transferred into sortilin/100 before regression analysis. After univariate analysis, multivariate logistic regression analysis was performed by the forward (LR) method. CAC, coronary artery calcification.

**Fig. 3.** Details of CCE. The bar chart (left) and Venn diagram (right) showed the number of patients who suffered from angina pectoris, myocardial infarction, congestive heart failure, transient ischemic attack, and stroke. CCE, cardiovascular and cerebrovascular events.
significantly higher than that in patients without CCE (603.3 ± 263.9 vs. 347.7 ± 169.5 pg/mL, p < 0.001) (Fig. 4).

As shown in Table 3, the multivariable Cox regression analysis showed that the risk of CCE in patients with sortilin ≥396 pg/mL was 8.588 times higher than that in patients with sortilin <396 pg/mL (95% CI: 1.919–38.43, p = 0.005). Compared with non-CAC patients, the risk of CCE in patients with CAC was 5.210 times higher (95% CI: 1.093–24.83, p = 0.038). Besides, diabetes mellitus was independently associated with the risk of CCE (HR 3.424; 95% CI: 1.348–8.701, p = 0.010). Before Cox analysis, all indicators were judged to meet the equal proportional hazards.

In addition, we proposed a cutoff value of 418 pg/mL for serum sortilin level, which was able to predict the occurrence of CCE with 75.0% sensitivity and 71.9% specificity. The area under the curve was 0.778 (95% CI: 0.673–0.883), as shown in Figure 5a. The percentage of CCE free of the serum sortilin subgroup in MHD patients is shown in Table 2.

| Parameter          | CCE (n = 28) | Non-CCE (n = 83) | p value |
|--------------------|-------------|-----------------|---------|
| Age, years         | 48 (44, 55) | 49 (40, 58)     | 0.983   |
| Male, n (%)        | 16 (57.14) | 44 (53.01)      | 0.827   |
| BMI, kg/m²         | 21.80 (19.45, 24.25) | 21.30 (20.00, 23.00) | 0.538   |
| Current smokers, n (%) | 10 (35.71) | 27 (32.53)      | 0.818   |
| Hypertension, n (%) | 25 (89.29) | 69 (83.13)      | 0.554   |
| DM, n (%)          | 9 (32.14)  | 11 (13.25)      | 0.043   |
| SBP, mm Hg         | 147 (136, 163) | 145 (133, 164)  | 0.659   |
| DBP, mm Hg         | 89±12      | 88±12           | 0.792   |
| Dialysis, months   | 40 (25, 64) | 24 (12, 38)     | 0.001   |
| Medication history, n (%) |           |                 |         |
| CCB                | 21 (75.00) | 65 (78.31)      | 0.795   |
| ACEI/ARB           | 9 (32.14)  | 26 (31.33)      | 0.990   |
| α,β-Blocker/α-blocker | 17 (60.71) | 53 (63.86)      | 0.823   |
| β-Blocker          | 14 (50.00) | 29 (34.94)      | 0.182   |
| Calcium supplement | 8 (28.57)  | 36 (43.37)      | 0.187   |
| Vitamin D analogs  | 22 (78.57) | 51 (61.45)      | 0.113   |
| Phosphate binders  | 14 (50.00) | 24 (28.92)      | 0.064   |
| Hb, g/L            | 99±14      | 95±18           | 0.311   |
| PLT, ×10⁹/L        | 150±40     | 159±51          | 0.400   |
| TG, mmol/L         | 1.18 (0.73, 2.15) | 1.26 (0.93, 1.79) | 0.796   |
| TC, mmol/L         | 4.34±1.18  | 4.05±1.13       | 0.247   |
| HDL-C, mmol/L      | 1.32±0.36  | 1.21±0.37       | 0.185   |
| LDL-C, mmol/L      | 2.59±0.99  | 2.34±0.82       | 0.188   |
| Ca, mmol/L         | 2.29 (2.10, 2.39) | 2.24 (2.08, 2.35) | 0.498   |
| P, mmol/L          | 2.09±0.47  | 1.89±0.50       | 0.056   |
| CaxP, mmol/L²      | 4.94±1.16  | 4.20±1.29       | 0.008   |
| PTH, pg/mL         | 450 (292, 727) | 289 (130, 483)  | 0.062   |
| hs-CRP, mg/L       | 2.69 (0.63, 8.28) | 1.30 (0.40, 7.42) | 0.292   |
| LVMI, g/m²         | 146±40     | 129±37          | 0.056   |
| LVH, n (%)         | 21 (75.00) | 33 (57.89)      | 0.154   |
| Carotid plaque, n (%) | 22 (78.57) | 22 (38.60)      | 0.001   |
| ValvC, n (%)       | 8 (28.57)  | 11 (19.30)      | 0.409   |
| AAC, n (%)         | 16 (57.14) | 18 (31.58)      | 0.034   |
| CAC, n (%)         | 22 (78.57) | 30 (52.63)      | 0.032   |

Continuous variables are shown as mean±standard deviation or median and interquartile range. Categorical variables are shown as counts and percentages. p values for unpaired t test or Mann-Whitney test or χ² test. Bold represents p < 0.05. CCE, cardiovascular and cerebrovascular events; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; Hb, hemoglobin; PLT, platelet; TG, triglyceride; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; PTH, parathyroid hormone; LVMI (left ventricular mass index) = LVM (left ventricular mass)/BSA (body surface area); LVH, left ventricular hypertrophy; ValvC, valve calcification; AAC, abdominal aortic calcification; CAC, coronary artery calcification.
in Figure 5b ($p < 0.001$). The Kaplan-Meier analysis showed a significant difference in the CCE occurrence in MHD patients with sortilin ≥390 pg/mL compared to sortilin ≤390 pg/mL MHD patients.

**Discussion**

Calcification of vessels, especially the coronary artery, is highly prevalent in MHD patients and has been associated with an increased CV risk as well as with all-cause mortality [21–23]. Although existing evidence hints toward the contribution of VC in CV mortality and CVD morbidity in dialysis recipients, there is still a need and interest to continuously determine and monitor VC’s burden and its risk factors. Several studies in cardiology suggest that sortilin may be related to VC and CV events, but none has done any relevant research in the field of kidney disease. Therefore, this study aimed to assess the potential role of serum sortilin in CAC and CCE, along with identifying other risk factors. We surprisingly found that serum sortilin level is positively correlated with CAC in MHD patients. Besides, we showed an incidence of new CCE of 25.2% in MHD patients during the 45-month follow-up, and serum sortilin was independently associated with the CCE.

In this study, we compared the data of the CAC-negative group and different degrees of the CAC-positive group first. We found that CAC was related to dialysis time, DM, blood phosphorus, calcium-phosphorus product, and carotid plaque. Nondialysis diabetes patients also have high CACs, which further confirmed that diabetes is a risk factor for CAC [24]. Hyperglycemia, hyperinsulinemia, oxidative stress, and abnormal adipokines may be the causes of diabetic vascular disease. Metabolic disorders of calcium and phosphorus are universal and crucial in VC of MHD patients. This study showed 22.1% of patients with serum phosphorus concentration >7.0 mg/dL (2.26 mmol/L) and 66.9% of patients with serum phosphorus concentration >5.5 mg/dL (1.78 mmol/L), which is consistent with the Chinese dialysis achievement and practice model study (DOPPS) published in 2019 [25]. DOPPS I and II studies have confirmed that phosphorus and calcium-phosphorus product were independent risk factors for CV events [26, 27]. All these results suggest that serum phosphorus is essential in VC and CV events, while serum phosphorus management in MHD patients in China is not ideal [25, 28]. Optimization of MBD management, especially phosphorus-related indicators, may have a good impact on the prognosis of MHD patients. However, AAC and ValvC did not show a linear trend with the aggravation of CAC, which was inconsistent with previous studies [20, 29, 30]. The reason for this is unclear. Further studies with a large number of patients will be done to explore this point.

Genome-wide association studies recently have shown a strong association between 1p13 locus and CVD [31–33]. The 1p13.3 locus contains 4 genes, CELSR2, PSRC1, MYBPHL, and SORT1. Sortilin encoded by the SORT1 gene is a multiligand sorting receptor, which has the functional characteristics of the vacuolar protein sorting 10 protein domain family. It is mainly located on the anti-Golgi network (TGN) and acts as a lysosome sorting receptor, which helps separate new synthetic protein cargo from the anti-Golgi network to the early endosome [34]. Sortilin can also transport soluble lysosomal proteins to lysosomes [35]. A small fraction of sortilin (about 10%) is located on the cell membrane and binds and internalizes various ligands through receptor-mediated endocytosis [36]. Emerging evidence suggests a significant role of sortilin in the pathogenesis of CVD and metabolic diseases, including type II diabetes mellitus by regulating insulin resistance [37, 38], atherosclerosis caused by arterial wall inflammation [9], dyslipidemia of lipoprotein metabolism, and VC [39–41].

Previous studies related to sortilin mainly focused on its lipid metabolic activity outside the brain. However, recent studies have shown that the effect of sortilin on
vascular inflammation and calcification might have nothing to do with lipids [9, 11, 42]. A clinical research of Goettsch et al. [39] demonstrated that sortilin serum levels were positively correlated with CRP, TC, and LDL-C levels, whereas when CRP, TC, or LDL-C levels were added to the multivariate model, along with other known AAC risk factors, the sortilin-AAC association remained significant. Furthermore, treatment with statins or fibrates did not alter the association between serum sortilin and AAC, suggesting that the observed association between high serum sortilin and severe AAC may not be associated with lipid abnormalities. This is in line with our results, which indicate that sortilin is an independent risk factor for CAC. Goettsch et al. [11] also reported reduced atherosclerotic VC in mice lacking sortilin, and they had demonstrated that sortilin is a crucial component of extracellular vesicles required for their calcification propensity. Besides, Sun et al. [40] showed that sortilin could mediate the aggregation of matrix vesicles in the early calcification stage. Hence, serum sortilin levels are likely to reflect the susceptibility to calcification.

There is increasing evidence suggesting that sortilin is independent of the CV effects of cholesterol. Ogawa et al. [43] showed that plasma sortilin levels had significant

Table 3. Cox regression analysis for influencing factors of CCE in MHD patients

| Variable                           | p value | Hazard ratio | 95% of hazard ratio |
|------------------------------------|---------|--------------|---------------------|
|                                    |         |              | lower | upper |
| Diabetes mellitus                  | 0.010   | 3.424        | 1.348 | 8.701 |
| Dialysis, months                   | 0.056   | 1.013        | 1.000 | 1.026 |
| Ca × P, mmol/L²                    | 0.647   | 0.902        | 0.578 | 1.405 |
| Sortilin                           | 0.005   | 8.588        | 1.919 | 38.43 |
| Carotid plaque                     | 0.120   | 2.301        | 0.804 | 6.580 |
| CAC                                | 0.038   | 5.210        | 1.093 | 24.83 |
| Abdominal aortic calcification     | 0.104   | 2.182        | 0.852 | 5.591 |

Before Cox analysis, all the indicators included in the regression analysis were judged to meet the equal proportional hazards. Sortilin was divided into 2 groups in the regression analysis: ≥396 or <396 pg/mL. CCE, cardiovascular and cerebrovascular events; MHD, maintenance hemodialysis; CAC, coronary artery calcification.

Fig. 5. Serum sortilin levels and CCE. a ROC curve analysis of the ability of sortilin to predict cardiovascular events. AUC = 0.778, p < 0.001. b Kaplan-Meier analysis of the percentage of CCE free in the serum sortilin subgroup of MHD patients. The percentage of CCE free is shown in the blue line for sortilin ≥390 pg/mL and the green line for sortilin <390 pg/mL in MHD patients. The time is expressed as months. Statistical significance was determined by the log-rank (Mantel-Cox) test. CCE, cardiovascular and cerebrovascular events; MHD, maintenance hemodialysis.
positive associations with CV risk factors: LDL-C, TG, and serum uric acid in patients with hypertension, dyslipidemia, and/or diabetes without coronary artery disease. One published study of the Framingham Heart Study [44] demonstrated that the neurotensin and pro-neurotensin receptor (sortilin receptor 1) is associated with an increased incidence of CV events. The study also reported that diabetes, dialysis time, and serum sortilin levels were independently associated with the occurrence of CCE. Besides, a study of 745 elderly community male residents showed that after adjusting for potential confounding factors (including CRP, TC, and LDL-C), high serum sortilin levels were associated with a 3-fold increase in the risk of adverse CCE, independent of traditional Framingham risk factors [39]. In conclusion, the intervention of sortilin may have a substantial impact on CVD since it acts at multiple levels simultaneously.

Our results are noteworthy because we included subjects who used strict criteria to minimize possible bias. We excluded subjects who were treated with statins. Preclinical in vivo evidence suggests that pitavastatin or pravastatin treatment for 8 months can reduce circulating sortilin levels by 8 and 16%, respectively [45]. Therefore, statins have an impact on circulating sortilin levels, and we eliminated this bias by including only those who did not receive statins [46].

Limitations

Our research has some limitations. First of all, we do not have data on the presence of sortilin SNPs. It has been reported that some SNPs in the 1p13.3 region, especially rs646776, rs599839, rs12740374, have a great impact on the expression of the SORT1 gene and the function of sortilin protein [32, 47]. Therefore, sortilin polymorphism may be associated with the occurrence of CV events. Second, this study is a single-center prospective study with small sample size. We did not make further analysis of CAC, AAC, and ValvC. Finally, the recommended threshold of sortilin for predicting CCE has relatively low sensitivity and specificity, so we cannot predict the occurrence of CCE only based on serum sortilin level. Large-scale prospective cohort studies are necessary to confirm the usefulness of circulating sortilin in predicting CCE and the possibility of combining other indicators to predict CCE.

Conclusion

Sortilin may be a newly found protein that is independently associated with CAC and CCE in MHD patients.

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Statement of Ethics

This study adhered to the principles of the Declaration of Helsinki II and was approved by the Medical Ethics Committee of Xiangya Hospital, Central South University (Ethical Code: 201512568). Written informed consent was obtained from all the study participants.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

Jie Xu participated in research design, data collection, statistical analysis, and manuscript drafting. Zhou Xiao and Qiong-Jing Yuan participated in data collection, Yang-Shuo Tang provided technical support such as B-ultrasound, and Qiao-Ling Zhou participated in the research design and article review and offered financial support. Chanjuan Shen, Joshua D. Ooi, and Yong Zhong were involved in data analysis and article review. All authors have read and approved the final version of the manuscript.

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