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Discovery of FLNA and VCAN as Novel Plasma Diagnostic Biomarkers for Sarcopenia in Older Chinese Adults

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Abstract

This study aimed to screen and verify plasma diagnostic biomarkers for sarcopenia in older Chinese adults using data-independent acquisition-based proteomics. We recruited community-dwelling older Chinese adults aged ≥ 65 years and divided them into sarcopenia and non-sarcopenia groups following the Asian Working Group for Sarcopenia 2019 diagnostic criteria. Plasma samples from six individuals in each group were subjected to proteomics screening to identify differentially expressed proteins. For validation using the enzyme-linked immunosorbent assay (ELISA), the sample size was increased to 52 in the sarcopenia group and 107 in the non-sarcopenia group. The diagnostic efficacy of candidate biomarkers was assessed using receiver operating characteristic curve analysis. A total of 48 differential proteins were identified (38 upregulated, 10 downregulated). ELISA confirmed that FLNA and VCAN were highly upregulated in the sarcopenia group and were independent risk factors for sarcopenia. FLNA showed better diagnostic efficacy (area

under the curve [AUC] = 0.797, 95% confidence interval (CI): 0.727–0.868) than VCAN (AUC = 0.654, 95% CI: 0.554–0.754). In conclusion, the serum levels of FLNA and VCAN are elevated in sarcopenia, and FLNA is a promising diagnostic biomarker for sarcopenia.

Keywords: sarcopenia; plasma biomarkers; DIA proteomics; Chinese community elderly; FLNA; VCAN

1. Background

Sarcopenia is a prevalent disease among older adults, characterised by an ageing-related decline in skeletal muscle mass and muscle strength [1]. According to a systematic review focused on older European adults, the prevalence of sarcopenia—defined per the diagnostic criteria of the European Working Group on Sarcopenia in Older People—ranged from 1% to 29%, with a prevalence of 11%–50% among people aged 80 years and above [2]. Following the 2014 criteria of the Asian Working Group for Sarcopenia (AWGS), epidemiological studies in some Asian countries have reported sarcopenia prevalence rates of 5.5%–25.7% [3]. Analysis of data from 6,172 individuals aged 60 years and older from the 2015 China Health and Retirement Longitudinal Study revealed that the overall prevalence of sarcopenia in China was 18.6%, as defined by the 2019 AWGS criteria, with sex-wise prevalence rates of 18.4% and 18.9% among men and women, respectively. Further, the prevalence was 9.4% in urban areas and 21.7% in rural areas, and it increased with age, from 11.5% in the 60–69 years age group to 27.2% and 52.0% in the 70–79 and ≥80 years age groups, respectively [4]. Another meta-analysis retrieved and analysed 58 studies on the prevalence of sarcopenia in Chinese people aged 60 years and older, reporting prevalence rates of 3.3%–45.7%. The considerable variance in the reported prevalence of sarcopenia can be attributed to differences between the studied regions and the ages of the older adults surveyed. Sarcopenia has an insidious onset, and the disease is often difficult to detect until the manifestation of adverse consequences. Moreover, sarcopenia is closely associated with poor prognosis in various malignant tumours, increasing the risk of dysphagia, cognitive impairment, fractures, falls, hospitalisations and all-cause mortality in older adults. Sarcopenia is also correlated with higher levels of albuminuria, depression and various metabolic diseases [5,6], severely diminishing the quality of life of older adults and imposing a heavy economic burden on families and the society [7]. Early diagnosis and intervention are paramount to improving the health of older adults with sarcopenia.

The pathogenesis of sarcopenia is complex, involving multiple dysfunctional processes, such as chronic inflammation, oxidative stress, mitochondrial dysfunction, lipotoxicity and imbalanced protein metabolism [8–10]. Currently, sarcopenia is diagnosed mainly through muscle mass measurements and physical function tests, but the requisite equipment are not widely available in community health centres and primary healthcare facilities. Moreover, some older adults cannot undergo physical function tests due to comorbidities. Together, these obstacles have delayed timely diagnosis and intervention in community-dwelling older adults with sarcopenia, exposing the urgent need for a screening and diagnostic method that can be easily disseminated to healthcare institutions and is suitable for older adults with various physical conditions. A potential solution lies in the measurement of serological markers in blood samples—a method that is both simple and feasible.

This study aimed to screen and verify potential plasma biomarkers for sarcopenia using quantitative proteomics analysis based on data-independent acquisition (DIA). Candidate biomarkers were validated using enzyme-linked immunosorbent assay (ELISA). The present findings can facilitate early detection of sarcopenia in older adults and support the implementation of early intervention programmes.

2. Results

2.1. Sample Screening and Research Process

In this study, we recruited older adults aged ≥ 65 years from the Dashimiao Town Central Health Station in Chengde City, Hebei Province. Individuals with missing data or who failed to meet the inclusion and exclusion criteria were excluded from the study ($n = 73$). A total of 513 individuals were enrolled (Figure 1), of which 53 (10.33%) were diagnosed with sarcopenia and 460 (89.67%) were not. Accordingly, the prevalence of sarcopenia in this cohort of older adults from the Dashimiao Community was 10.33%.

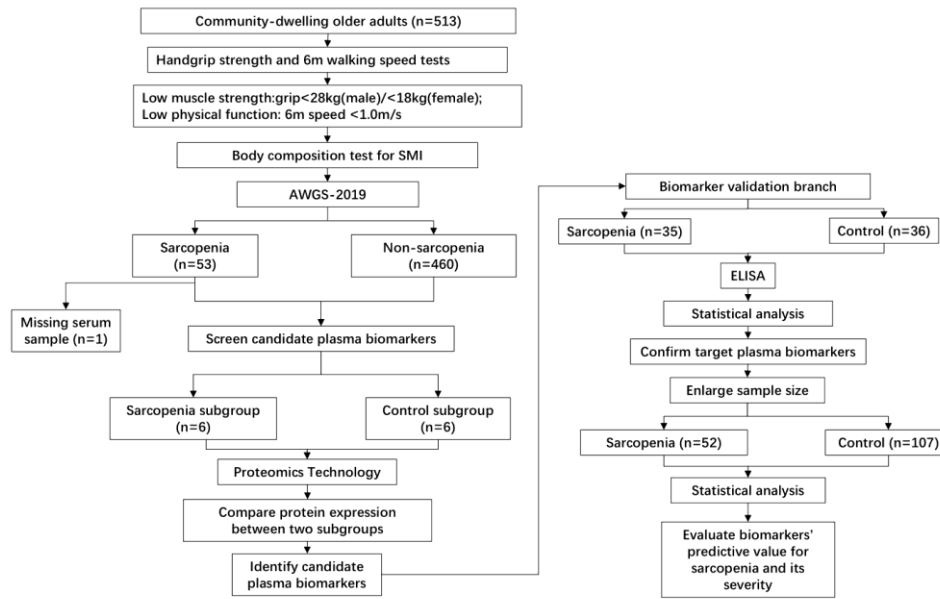


Figure 1. Schematic workflow of the study. ELISA, enzyme-linked immunosorbent assay; AWGS-2019, Asian Working Group for Sarcopenia 2019; SMI, skeletal muscle mass index.

2.2 Baseline Characteristics of Participants

We first analysed the general characteristics of samples from the discovery cohort. No significant differences were observed in age and gender composition, liver and kidney function, smoking and alcohol consumption history and diabetes and hypertension history between the sarcopenia and non-sarcopenia groups (all $P > 0.05$; Table 1).

Table 1. Baseline characteristics of groups in the DIA analysis and discovery cohorts.

Variable	DIA analysis cohort			Discovery cohort	
	Sarcopenia (n = 6)	Non-sarcopenia (n = 6)	<i>p</i>	Sarcopenia (n = 35)	Non-sarcopenia (n=36)
Age	76.67±6.653	73.17±4.355	0.306	73.00(71.00,77.00)	71.50(70.00,75.75)
Sex (n,%)					
Female	3(50)	3(50)		16(45.7)	17(47.2)
Male	3(50)	3(50)	1.000	19(54.3)	19(52.8)
Height (cm)	147.500±9.160	161.833±7.782	0.015	153.000(149.250,160.250)	161.500(156.250, 168.000)
Weight (kg)	48.950±10.544	59.867±6.176	0.053	50.618±5.977	66.703±10.102
BMI (kg/m ²)	23.650(18.925,24.425)	22.400(20.975,24.350)	0.810	20.400(19.200,22.200)	23.000(22.350,24.950)
ALT (U/L)	18.000±3.521	17.667±3.670	0.876	18.206±5.682	18.917±5.304
AST (U/L)	24.500(22.750,26.000)	25.500(21.250,30.250)	0.901	23.941±5.856	22.083±3.628
Cr (μmol/L)	70.167±16.905	71.167±9.152	0.127	65.000(57.750,87.250)	74.000(69.000,81.000)
Urea (mmol/L)	6.385(6.088,7.708)	6.105(5.620,6.910)	0.262	5.981±1.883	5.628±1.080
SMI (kg/m ²)	5.667±0.739	7.225±0.519	0.007	5.600(5.300,6.700)	6.800(6.300,7.400)
6-m gait speed (m/s)	0.785±0.256	1.007±0.153	0.099	0.857(0.757,0.946)	1.090(1.000,1.200)
Handgrip strength (kg)	17.817±4.418	26.600±5.784	0.014	19.700(14.500,26.500)	25.300(21.025,33.225)
CC (cm)	29.400±1.570	32.967±2.414	0.020	30.388±1.916	34.719±2.962
Smoking (n,%)	0(0)	1(16.7)	0.296	8(22.9)	4(11.1)

Alcohol consumption (n,%)	1(16.7)	2(33.3)	0.505	7(20)	11(30.6)
Diabetes mellitus (n,%)	0(0)	0(0)	1	5(14.7)	1(2.8)
Hypertension (n,%)	6(100)	3(50.0)	0.046	19(55.9)	21(58.3)

DIA, Data-independent acquisition; BMI, body mass index; SMI, skeletal muscle mass index; CC, calf circumference; ALT, alanine transaminase; AST, aspartate transaminase; Cr, serum creatinine. Normally distributed continuous data were presented as mean $\pm$ standard deviation and compared using independent Student's t-test; non-normal continuous variables were expressed as median (interquartile range) and analysed by Mann-Whitney U test. Categorical variables (smoking, alcohol consumption, chronic diseases) were compared using Chi-square test or Fisher's exact test as appropriate.

2.3 Differentially Expressed Proteins

The results of principal component analysis are shown in Figure 2a. All samples fell within the 95% confidence interval (Hotelling's T^2 ellipse) with no outliers detected, demonstrating that the experimental data were stable and reliable. Samples from the two groups clustered separately on the principal component analysis score plot, indicating highly consistent and reproducible protein expression patterns among intragroup samples. They exhibited partial separation along the PC1 axis, with PC1 and PC2 explaining 24.6% and 10% of the total variance, respectively. This clustering pattern revealed remarkable overall differences in global protein expression profiles between the two groups, laying a solid foundation for subsequent screening of differentially expressed proteins and functional analyses.

The results of volcano plot analysis are presented in Figure 2b. Based on the thresholds of $P < 0.05$ and $|\log_2(\text{fold-change})| \geq 0.263$ (corresponding to fold-change ≥ 1.2 or fold-change ≤ 0.83), a total of 48 significantly differentially expressed proteins were identified, including 38 upregulated and 10 downregulated proteins. The greater number of significantly upregulated proteins indicates extensive activation of protein expression in the sarcopenia group compared with the non-sarcopenia group. Several upregulated proteins exhibited extremely high fold-changes in expression and may exert crucial regulatory effects on the phenotypic differences between the two groups.

Hierarchical clustering analysis was performed on the differentially expressed proteins, and the results were visualised (Figure 2c). Twelve samples were clustered into two major branches corresponding to the sarcopenia and non-sarcopenia groups, verifying the accuracy of the grouping and the reliability of the differential screening results. The differentially expressed proteins exhibited distinct coexpression patterns: highly expressed proteins (red blocks in Figure 2c)

and low-expression proteins (blue blocks in Figure 2c) in the sarcopenia group clustered together, suggesting that functionally related proteins collaboratively regulate the phenotypic differences between the two groups.

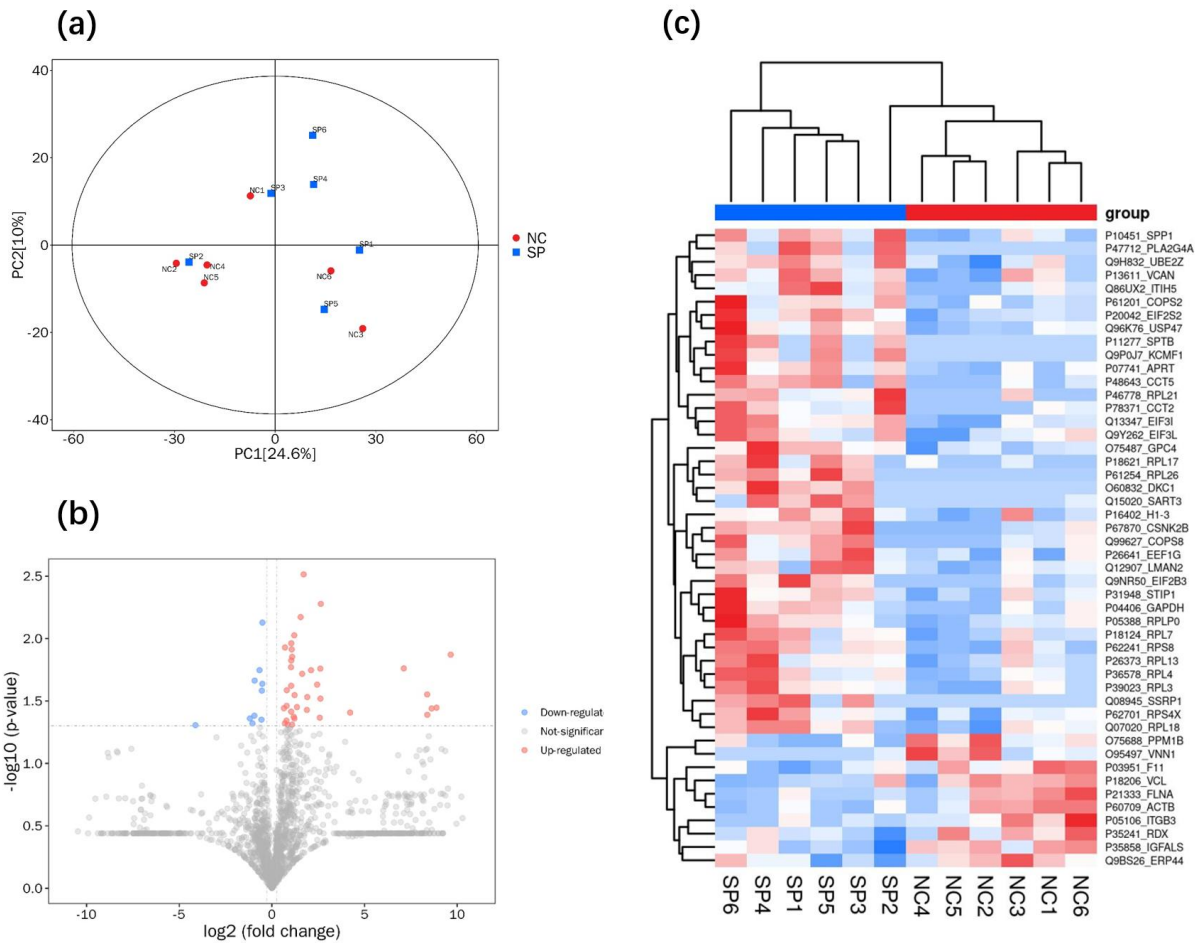


Figure 2. Proteomic analysis of plasma proteins in sarcopenia patients and non-sarcopenic controls in the discovery cohort. **(a)** Principal component analysis plot of all detected proteins. **(b)** Volcano plot of differentially expressed proteins; blue dots represent down-regulated proteins, red dots represent upregulated proteins. **(c)** Heatmap of significantly altered proteins between the sarcopenia (SP) and non-sarcopenic control (NC) groups.

Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis revealed that the differentially expressed proteins were significantly enriched in the ribosome pathway (hsa03010; Figure 3a). Additionally, enrichment was noted in pathways such as coronavirus disease 2019, focal adhesion and muscle cell cytoskeleton. The results of Gene Ontology enrichment analysis (Figure 3b) indicated that the differentially expressed proteins were mainly enriched in (i) metabolic processes of organic nitrogen compounds, protein metabolism, gene expression, trans-

lation and peptide biosynthesis in the biological process domain; (ii) extracellular exosomes, extracellular vesicles, ribosomal subunits and ribonucleoprotein complexes in the cellular component domain; (iii) RNA binding, nucleic acid binding and ribosome structural composition in the molecular function domain.

Integrating the results of the three different enrichment analyses, we found that the differentially expressed proteins between the two groups were highly concentrated in protein translation and synthesis pathways, indicating that changes in cellular translation activity may be a core biological difference between the sarcopenia and non-sarcopenia groups.

Protein–protein interaction network analysis (Figures 3c and 4) revealed that eukaryotic translation initiation factor 2 subunit β (EIF2S2) interacted with the largest number of genes/proteins ($P = 0.00673$), with a total of 16 interacting partners: CSNK2B, RPL17, RPLP0, RPS8, RPL18, RPL26, EIF3L, RPL13, RPL4, EEF1G, RPL7, RPL3, RPL21, EIF2B3, EIF3I and RPS4X.

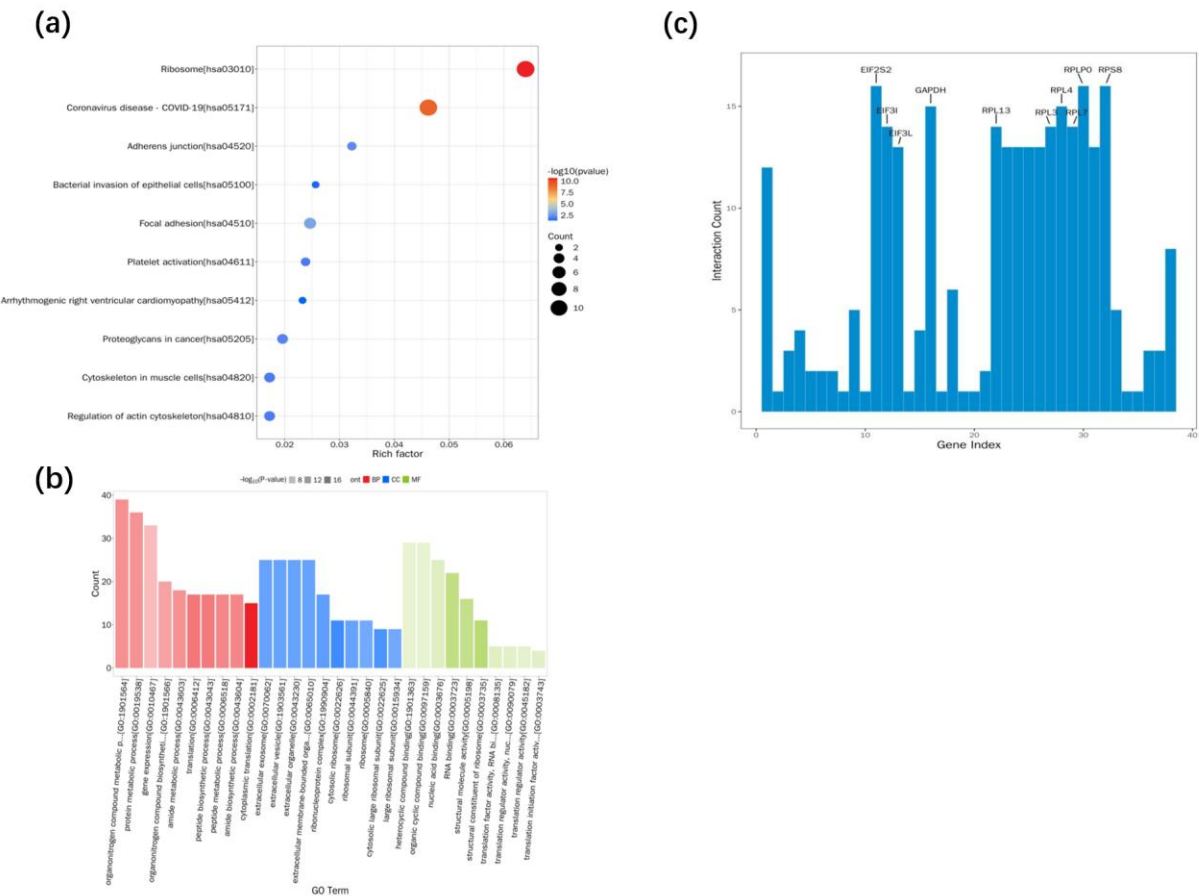


Figure 3. Functional enrichment and protein–protein interaction analysis of differentially expressed proteins. (a) Bubble plot depicting the results of Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis, coloured by P values and sized by gene

count. **(b)** Bar chart of top-level Gene Ontology biological process terms ranked by gene count. **(c)** Bar graph of the top enriched hub genes in the protein–protein interaction network.

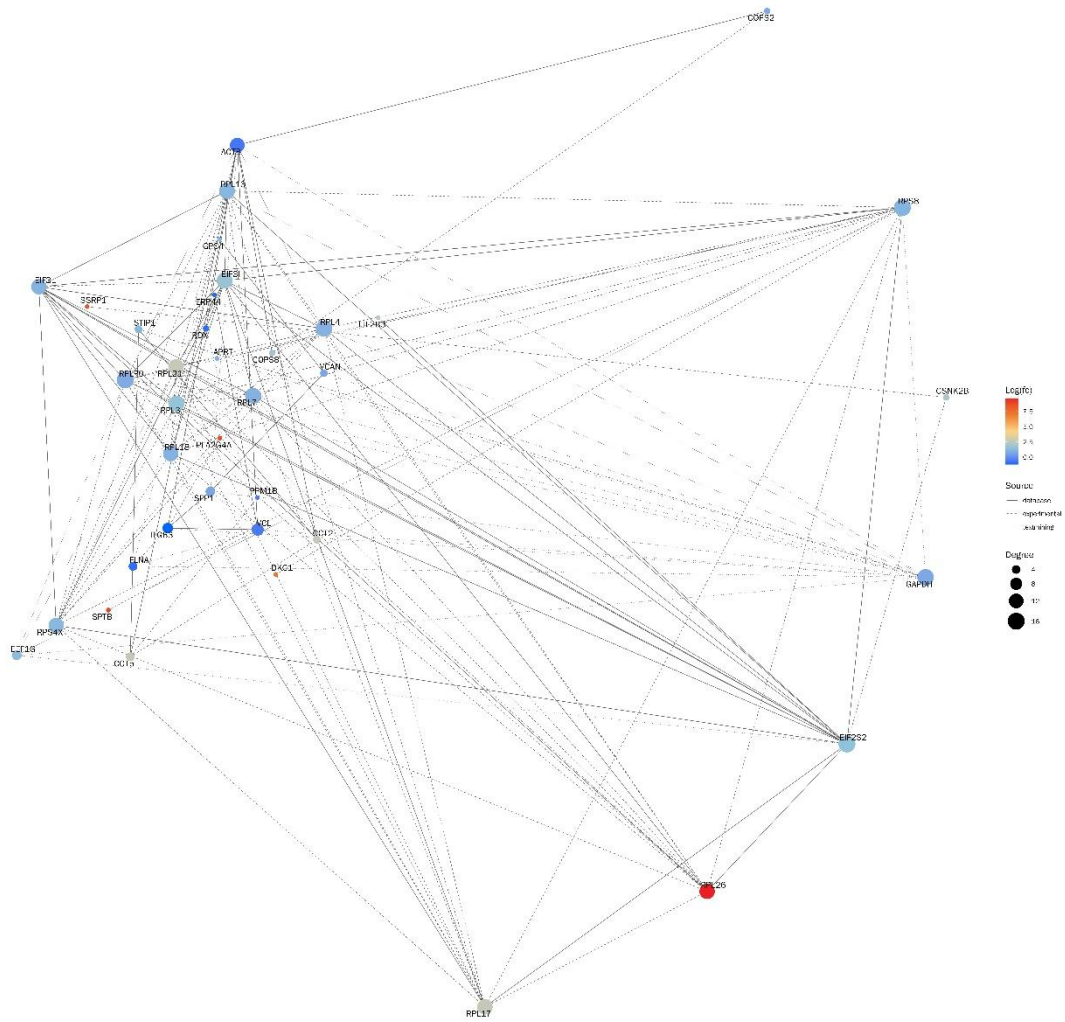


Figure 4. Protein–protein interaction network of differentially expressed proteins between the non-sarcopenia and sarcopenia groups. Node colour represents the $\log_2(\text{fold-change})$ of proteins; node size corresponds to the degree value. Different line types indicate diverse interaction evidence, including text mining, experimental and coexpression support.

EIF2S2 belongs to the eukaryotic initiation factor family and acts as a core regulatory protein during translation initiation. Its primary functions include mediating the binding of mRNA to the small ribosomal subunit, triggering the first step of protein synthesis; modulating translation initiation efficiency and directly governing the global rate of intracellular protein synthesis; and responding to cellular stress signals (including nutrient deprivation, inflammation and oxidative stress) and maintaining cellular homeostasis by regulating translation initiation.

After screening the literature and filtering according to the known biological functions of proteins, we selected GADPH, ITGB3, VCL, CCT5, GPC4, VNN1, ITIH5, ACTB, filamin A (FLNA), versican (VCAN) and SPP1 from the list of differentially expressed proteins as candidate biomarkers for sarcopenia.

2.4 Validation of Serum Biomarkers for Sarcopenia

The serum concentrations of the selected candidate biomarkers were measured using ELISA. The results demonstrated that the following proteins level of ACTB were significantly elevated in the sarcopenia group compared with the non-sarcopenia group: ACTB (1.422 [1.102, 1.713] vs. 0.747 [0.545, 1.531], $p < 0.001$); FLNA (103.250 [73.750, 199.100] vs. 80.325 [54.900, 105.938], $p = 0.001$); VCAN (150.160 [118.080, 201.440] vs. 108.440 [94.440, 133.700], $p = 0.002$); and SPP1 (2.547 ± 2.467 vs. 0.804 ± 0.874 , $p = 0.012$). No significant differences were observed in the expression of the other proteins between the sarcopenia and non-sarcopenia groups (Figure 5).

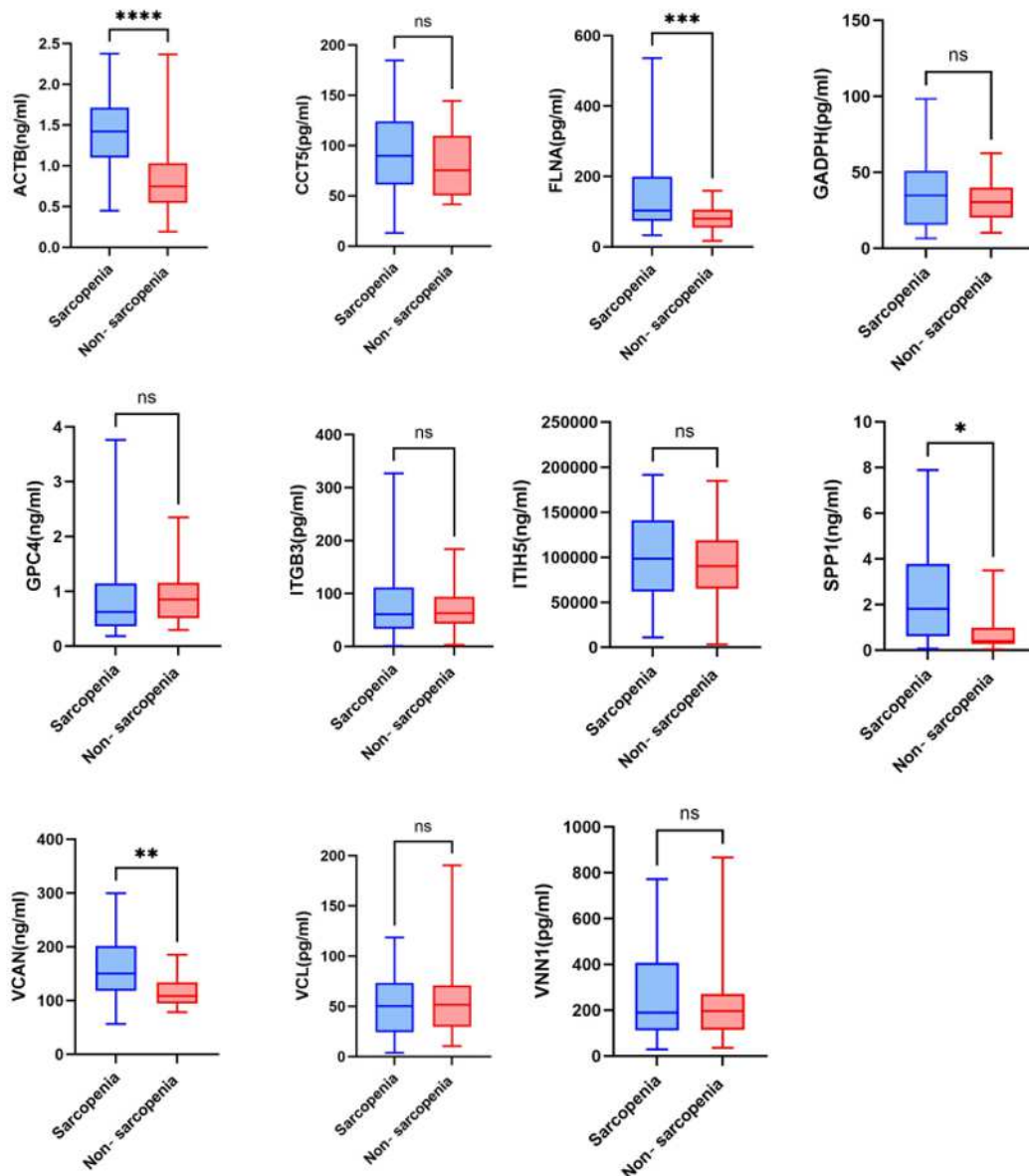


Figure 5. Candidate plasma biomarker concentrations in the sarcopenia and non-sarcopenic control groups in the discovery cohort. Box plots comparing the relative expression of candidate plasma biomarkers between the sarcopenia and non-sarcopenic control groups in the discovery cohort. Data represent the median and interquartile range. Statistical differences were determined using the Mann–Whitney U test. ns, no significant difference; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

2.4.1 Validation of FLNA and VCAN as diagnostic readouts for sarcopenia

The sample size was increased for validation analysis, with 52 individuals in the sarcopenia group and 107 in the non-sarcopenia group. The two groups did not differ significantly in age and gender distribution, but body mass index (BMI), skeletal muscle mass index (SMI), appendicular skeletal muscle mass and lean body mass were significantly lower in the sarcopenia group relative to the non-sarcopenia group ($p < 0.001$; Table 2).

Table 2. Baseline Characteristics of Two Groups in validation cohort.

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Variable		Sarcopenia (n = 52)	Non-sarcopenia (n = 107)	<i>p</i>
Age		75.94±6.111	74.93±3.013	0.265
Sex (n,%)	Male	24(46.2)	50(46.7)	0.946
	Female	28(53.8)	57(53.3)	
Height (cm)	Male	161.326±5.208	166.184±5.851	0.001
	Female	150.250±6.305	155.464±4.694	<0.001
Weight (kg)	Male	55.043±3.974	68.241±9.134	<0.001
	Female	47.750(44.000,51.000)	61.500(57.000,67.000)	<0.001
BMI (kg/m ²)	Male	20.488±2.083	23.821±3.255	<0.001
	Female	21.382±2.115	25.683±3.489	<0.001
SMI (kg/m ²)	Male	6.550(6.225,6.775)	7.400(7.200,7.850)	<0.001
	Female	5.300(5.100,5.500)	6.400(6.025,6.800)	<0.001
Soft Lean Mass (kg)	Male	39.392±3.357	45.286±3.628	<0.001
	Female	29.336±2.225	36.050±3.735	<0.001
Appendicular Lean Mass (kg)	Male	17.365±1.899	20.570±1.916	<0.001
	Female	11.631±1.325	15.591±1.996	<0.001
Percent Body Fat (%)	Male	23.442±7.064	25.741±7.444	0.234
	Female	33.575±6.655	37.792±5.847	0.005
ALT (U/L)		18.529±5.562	19.333±5.742	0.411
AST (U/L)		23.118±5.505	22.673±6.824	0.614
Cr (μmol/L)		72.706±19.159	75.058±13.379	0.376
Urea (mmol/L)		6.207±1.777	6.023±1.493	0.501
6-m gait speed (m/s)	Male	0.868±0.202	1.039±0.253	0.005
	Female	0.785±0.157	0.957±0.220	0.001
Handgrip strength (kg)	Male	24.582±6.185	30.466±7.202	0.001
	Female	14.550(11.400,18.025)	19.700(15.700,21.950)	<0.001
CC (cm)	Male	30.833±1.539	35.186±2.516	<0.001
	Female	29.746±1.906	34.080±2.554	<0.001
Smoking (n,%)	Male	9(37.5)	14(28.0)	0.408
	Female	1(3.6)	1(1.8)	0.603
Alcohol consumption (n,%)	Male	6(25.0)	26(52.0)	0.028
	Female	3(10.7)	10(17.5)	0.411
Diabetes mellitus (n,%)	Male	1(4.3)	6(12.0)	0.302
	Female	5(17.9)	6(10.5)	0.344
Hypertension (n,%)	Male	10(43.5)	28(56.0)	0.320
	Female	17(60.7)	37(64.9)	0.705
Fatty liver disease (n,%)	Male	2(8.7)	15(30.0)	0.045
	Female	4(14.3)	31(54.4)	<0.001

BMI, body mass index; SMI, skeletal muscle mass index; CC, calf circumference. All anthropometric and biochemical indicators were stratified by gender for subgroup comparison. Continuous variables are presented as mean ± standard deviation (normal distribution) or median (interquartile range) (skewed distribution). Intergroup differences were analysed using independent Student's t-test or Mann-Whitney U test; categorical variables were analysed using Chi-square test.

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In the validation cohort, the sarcopenia group exhibited significantly elevated expression of FLNA (102.475 [75.75, 187.375] vs. 54.600 [28.435, 87.900], $p < 0.001$) and VCAN (183.480 [130.120, 231.860] vs. 145.040 [120.000, 174.880], $p = 0.002$) compared with the non-sarcopenia group, but the significant difference in expression of ACTB and SPP1 between the two groups (noted in the discovery cohort) was lost (Figure 6).

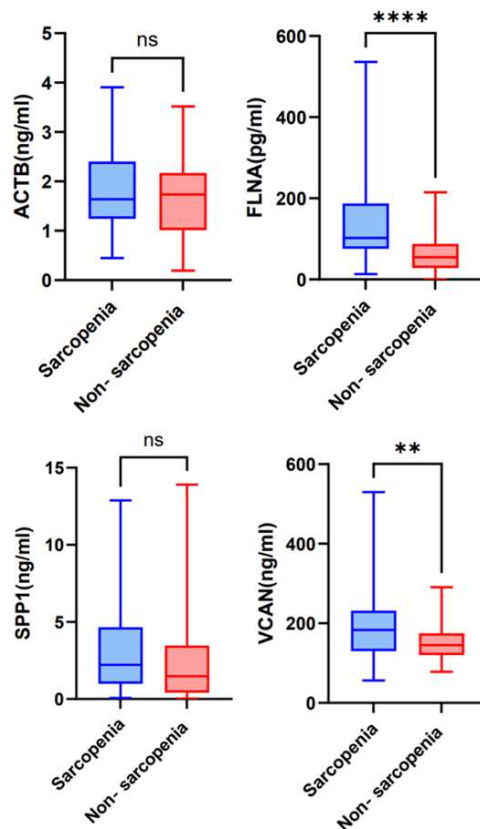


Figure 6. Comparison of protein plasma levels between the sarcopenia and non-sarcopenia groups in the validation cohort.

The proteins analysed were ACTB, FLNA, SPP1 and VCAN. Box plots display the median and interquartile range of protein concentrations. Statistical analysis was performed using the Mann–Whitney U test. ns, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

To verify the robustness of our findings, we examined the associations of FLNA and VCAN with sarcopenia-related anthropometric and body composition parameters in the validation cohort (Table 3). In the overall validation cohort, FLNA was inversely correlated with BMI ($r_s = -0.242$, $p = 0.004$) and SMI ($r_s = -0.214$, $p = 0.012$), whereas VCAN exhibited no significant associations with any body composition parameter. Among men in the cohort, FLNA was inversely

correlated with SMI ($r_s = -0.348$, $p = 0.006$) and appendicular lean mass ($r_s = -0.260$, $p = 0.043$), while VCAN was inversely correlated with SMI ($r_s = -0.366$, $p = 0.004$), soft lean tissue mass ($r_s = -0.386$, $p = 0.002$) and appendicular lean mass ($r_s = -0.353$, $p = 0.005$). Among women in the cohort, FLNA was negatively associated with BMI ($r_s = -0.286$, $p = 0.012$), SMI ($r_s = -0.416$, $p < 0.001$), soft lean tissue mass ($r_s = -0.414$, $p < 0.001$) and appendicular lean mass ($r_s = -0.412$, $p < 0.001$), whereas VCAN was not significantly correlated with any body composition parameter.

Table 3. FLNA and VCAN correlations with body composition, gait speed, handgrip strength: sex-stratified and overall analyses

Variable	FLNA						VCAN					
	Total		Male		Female		Total		Male		Female	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
BMI(kg/m ²)	-0.242	0.004	-0.124	0.342	-0.286	0.012	-0.106	0.216	-0.155	0.234	-0.013	0.909
SMI(kg/m ²)	-0.214	0.012	-0.348	0.006	-0.416	< 0.001	-0.124	0.148	-0.366	0.004	-0.200	0.083
Soft Lean Mass (kg)	-0.145	0.090	-0.213	0.100	-0.414	< 0.001	-0.119	0.164	-0.386	0.002	-0.224	0.052
Appendicular Lean Mass(kg)	-0.155	0.070	-0.260	0.043	-0.412	< 0.001	-0.112	0.194	-0.353	0.005	-0.220	0.056
Percent Body Fat(%)	-0.128	0.135	0.025	0.847	-0.100	0.392	-0.017	0.846	0.069	0.596	0.133	0.251
6m gait speed(m/s)	-0.102	0.206	-0.103	0.384	-0.139	0.215	-0.303	< 0.001	-0.279	0.016	-0.347	0.001
Handgrip strength(kg)	0.018	0.827	-0.100	0.401	-0.154	0.165	-0.095	0.239	-0.257	0.029	-0.329	0.002

r_s = Spearman correlation coefficient; p = statistical P value. Correlations of FLNA and VCAN with body composition, muscle function and gait parameters were analysed in the overall and sex-stratified cohort. Negative r_s values represent inverse correlations, while positive r_s values represent positive correlations.

Binary logistic regression analysis (Table 4) showed that in Model 1 (adjusted for age and sex), the risk of developing sarcopenia significantly increased for each one-unit increase in FLNA (OR = 1.020, $p < 0.05$) and VCAN (OR = 1.011, $p < 0.05$). As the number of adjustment variables increased, the independent predictive value of VCAN disappeared in Model 2 (further adjusted for body fat percentage), whereas that of FLNA remained significant in all models. In the fully adjusted Model 3, which accounts for age, sex, BMI and body fat percentage, FLNA remained an independent risk factor for sarcopenia (OR = 1.024, 95% CI: 1.010–1.038, $p < 0.001$).

Table 4. Binary logistic regression for associations between FLNA, VCAN and sarcopenia with sequential covariate adjustment.

Model	FLNA		VCAN	
	OR (95% CI)	p	OR (95% CI)	p
Model 1	1.020 (1.012, 1.029)	<0.001	1.011 (1.004, 1.018)	0.003
Model 2	1.026 (1.014, 1.039)	<0.001	1.010 (1.000, 1.019)	0.043
Model 3	1.024 (1.010, 1.038)	<0.001	1.002 (0.992, 1.013)	0.655

OR = odds ratio; 95% CI = 95% confidence interval.

Model 1: Adjusted for age and sex.

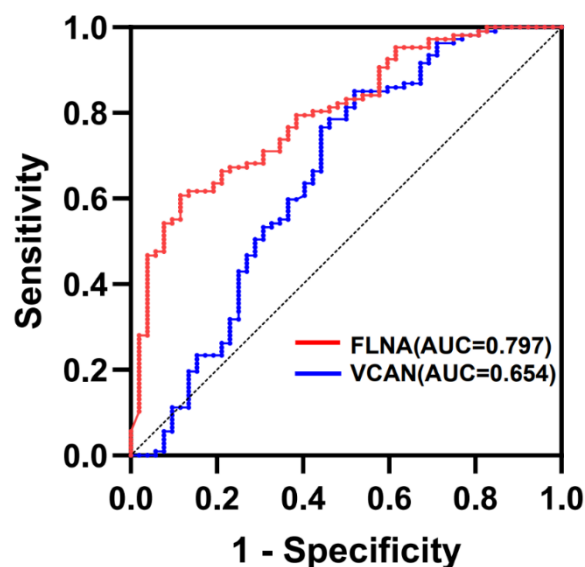
Model 2: Adjusted for all variables in Model 1 plus BMI.

Model 3: Adjusted for all variables in Model 2 plus percent body fat.

The diagnostic value of FLNA and VCAN for sarcopenia was further assessed through receiver operating characteristic curve analysis. Serum FLNA exhibited strong diagnostic performance for sarcopenia, with an area under the curve (AUC) of 0.797 (95%CI: 0.727–0.868), optimal cut-off value of 65.400 pg/mL, sensitivity of 88.5% and specificity of 60.7%. By contrast, serum VCAN showed limited diagnostic efficacy for sarcopenia; its AUC was 0.654 (95%CI: 0.554–0.754), accompanied by low sensitivity (48.1%) but high specificity (85.0%; Table 5).

Table 5. ROC curve of serum FLNA and VCAN for diagnosing sarcopenia.

Variable	AUC (95%CI)	Cut-off value	Sensitivity (%)	Specificity (%)	Youden's index	p
FLNA	0.797 (0.727–0.868)	65.400	88.500	60.748	0.492	<0.001
VCAN	0.654 (0.554–0.754)	190.640	48.100	85.047	0.331	0.002



The optimal cut-off values for FLNA and VCAN with their respective sensitivity, specificity and Youden's index are listed in Table

5. AUC, area under the curve.

3. Discussion

In this study, we combined DIA proteomics with ELISA validation to systematically screen and verify plasma protein biomarkers associated with sarcopenia among community-dwelling older Chinese adults. A total of 48 differentially expressed proteins were identified via proteomic analysis. Subsequent ELISA validation revealed that FLNA and VCAN were significantly upregulated in the sarcopenia group, among which FLNA emerged as an independent risk factor for sarcopenia (OR = 1.024, 95% CI: 1.010–1.038, $P < 0.001$) and exhibited favourable diagnostic performance (AUC = 0.797). Our findings highlight the potential of FLNA as a serological biomarker for sarcopenia screening in community-dwelling older adults.

FLNA is an actin-binding protein involved in cytoskeletal assembly and remodelling. It mediates the orthogonal branching of F-actin and the formation of stress fibres, thereby maintaining cellular morphology. FLNA regulates cell adhesion, migration and invasion, and acts as a scaffold protein participating in the transduction of multiple signalling pathways, including the cyclic AMP/protein kinase A, mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) and phosphoinositide-3-kinase/Akt pathways. FLNA also modulates membrane receptor recycling, vesicle trafficking and the formation of endoplasmic reticulum–plasma membrane contact sites and participates in the regulation

of cell cycle progression, cell proliferation and differentiation. Moreover, FLNA strengthens cell–matrix adhesion and protects cells against mechanical stress–induced apoptosis [11]. It also contributes to the development of the central nervous system and cardiovascular system [12,13].

This study found that serum FLNA concentration in the sarcopenia group was higher than that in the control group. After adjusting for confounding factors such as age, sex, BMI and body fat percentage, FLNA remained an independent risk factor for sarcopenia, and increase in FLNA expression was closely related to decrease in muscle mass. A previous study performed RNA sequencing on peripheral blood mononuclear cells, revealing significantly elevated FLNA mRNA expression in individuals with sarcopenia [14]. Proteomic analysis in the present study further confirmed the upregulation of FLNA at the protein level in patients with sarcopenia. The findings were verified in a larger validation cohort using ELISA. The results underscored the diagnostic value of FLNA for sarcopenia, with an AUC of 0.797, sensitivity of 88.5% and specificity of 60.7%.

VCAN is an extracellular matrix proteoglycan that contributes to disease progression by regulating cell proliferation, migration, adhesion and matrix assembly. VCAN contributes to the formation of the pericellular matrix, modulates mechanical signal transduction, regulates immune inflammatory responses, mediates leukocyte adhesion and release of proinflammatory cytokines and participates in tissue remodelling via its degraded fragments [15]. Wang J reported marked upregulation of VCAN in fibroblasts isolated from mice with pulmonary fibrosis. Fibroblast-specific knockout of VCAN significantly attenuated fibroblast activation and severity of pulmonary fibrosis in these mice [16]. Jiménez-Suárez J demonstrated that VCAN acts as a key downstream effector of the ERK5/mitogen-activated protein kinase 5 signalling pathway. Myocyte enhancer factor 2 (MEF2) belongs to a family of transcription factors involved in the morphogenesis of skeletal, cardiac and smooth muscle cells. ERK5 activates MEF2C through phosphorylation of serine and threonine residues within its transcriptional activation domain. The ERK5–MEF2 axis is essential for myotube maturation and governs the expression of myogenic differentiation 1, actin and myosin [17]. The ERK5–MEF2 pathway in soft tissue sarcomas contributes to tumour initiation and maintenance by regulating cell proliferation, migration, adhesion and tumorigenicity *in vivo* [18]. Previous studies have reported significantly elevated expression of VCAN in small extracellular vesicles isolated from the plasma of patients with sarcopenia; however, subsequent ELISA validation and evaluation of diagnostic performance were not undertaken [19]. Consistent with the proteomic findings in the present

study, VCAN was upregulated in the sarcopenia group, and this was subsequently confirmed through ELISA. Binary logistic regression analysis further revealed an association between VCAN and sarcopenia risk. However, the statistical significance of VCAN was lost after adjustment for body fat percentage, indicating that VCAN is not an independent risk factor for sarcopenia, and that its effect may be driven by fat mass. This study found that serum VCAN has some diagnostic value for sarcopenia, with an AUC of 0.654, sensitivity of 48.1% and specificity of 85.0%.

SPP1, also known as osteopontin, is a highly glycosylated and phosphorylated acidic protein expressed in various cells. SPP1 regulates the dynamic balance between bone resorption and bone formation in osteoporosis, promotes cartilage degeneration and inflammation in osteoarthritis and drives tumour progression through pathways such as the phosphoinositide-3-kinase/Akt/mammalian target of rapamycin pathway [20]. In an adenine-induced mouse model of chronic kidney disease-associated sarcopenia, integrated multiomics sequencing of serum, kidney and skeletal muscle proteins led to the identification of renal secretory SPP1. Through *in vitro* experiments on C2C12 myotubes and *in vivo* intervention with SPP1 neutralising antibody, the authors verified that injured kidneys synthesise and secrete SPP1 into the bloodstream. SPP1 upregulates muscle atrophy markers such as Murf-1 and disturbs pathways related to muscle energy metabolism and the extracellular matrix, thereby triggering skeletal muscle atrophy. Blocking circulating SPP1 significantly alleviates muscle atrophy and improves muscle strength in mice, confirming that circulating SPP1 derived from the kidney plays a key role in inducing skeletal muscle atrophy in chronic kidney disease [21].

ACTB, also known as β -actin, is a highly conserved housekeeping protein. ACTB is primarily involved in formation of F-actin filaments, maintenance of cellular morphology, cell adhesion, cell migration, intracellular vesicle transport, cell division and pseudopodium formation. In a study investigating *STAT3* as a common pathogenic gene in postmenopausal osteoporosis and sarcopenia, screening using four topological algorithms identified *ACTB* as a shared hub gene [22]. A study on amyotrophic lateral sclerosis (ALS) identified ACTB as a candidate antigen for ALS using serological analysis of recombinant cDNA expression libraries. Higher levels of anti-ACTB antibodies were associated with greater disability, later clinical stage and longer disease duration in patients with ALS. However, this study did not measure serum ACTB levels [23].

In our study, SPP1 and ACTB showed significant differences in expression between the sarcopenia and non-sarcopenia groups in the smaller discovery cohort, but these differences were not retained in larger-scale, gender- and age-matched

samples. As an inflammatory and metabolism-related protein, the serum concentration of SPP1 is easily affected by various comorbidities, such as pathogenic infections, osteoporosis and osteoarthritis. As a cytoplasmic skeletal protein, the serum level of ACTB is greatly influenced by pre-analytical factors, such as sample haemolysis and platelet activation. These nonspecific factors may dilute the true differences between the study groups. SPP1 has been confirmed to play a pathogenic role in chronic kidney disease-related sarcopenia, and ACTB autoantibodies have been shown to be related to ALS severity, highlighting the potential role of both these proteins in specific pathological conditions. Future studies should evaluate the true association of SPP1 and ACTB with sarcopenia under conditions of strictly matched confounding factors.

The present study has some limitations. First, all participants were recruited from a single community, which limits the representativeness of the sample. Multicentre trials recruiting populations from diverse regions are required to verify the generalisability of our conclusions. Second, the sample size in the proteomic screening phase was relatively small (n = 6 per group), which may have led to the omission of some differentially expressed proteins. Future studies should employ larger samples for screening or combine multiomics analyses.

In conclusion, through proteomics combined with ELISA validation, this study found that the serum levels of FLNA and VCAN were significantly elevated in patients with sarcopenia among a population of older Chinese adults. FLNA was positively correlated with sarcopenia and emerged as an independent risk factor with high diagnostic value. Serum VCAN also exhibited some diagnostic value for sarcopenia, with an AUC, sensitivity and specificity of 0.654, 48.1% and 85.0%, respectively. This study provides new candidate biomarkers for early serological screening of sarcopenia and lays an experimental foundation to enable further exploration of its pathogenesis.

4. Methods

4.1 Study Population

In this cross-sectional study, participants were recruited from free physical examinations of individuals aged ≥65 years from 10 villages under the jurisdiction of Dashimiao Town Central Health Centre in Chengde City in April and May, 2025. The participants were selected on the basis of inclusion and exclusion criteria. All participants completed basic demographic questionnaires and signed informed consent forms as well as personal privacy confidentiality agreements.

Following the AWGS 2019 criteria for diagnosing sarcopenia, we divided the participants into two groups, namely the sarcopenia group (n = 53) and the non-sarcopenia group (n = 460). Serum specimens were randomly chosen from both groups (n = 6 per group) and subjected to DIA-based quantitative proteomics to screen differentially expressed proteins and conduct subsequent bioinformatics analyses. The sample sizes were increased to n = 35 in the sarcopenia group and n = 36 in the non-sarcopenia group to measure the serum levels of differentially expressed proteins using ELISA. Finally, the candidate biomarkers were validated as diagnostic readouts for sarcopenia using a validation cohort comprising 52 individuals in the sarcopenia group and 107 age- and gender-matched participants in the non-sarcopenia group.

4.1.1 Inclusion and exclusion criteria

Inclusion criteria: age ≥ 65 years and meeting the AWGS 2019 diagnostic criteria for sarcopenia.

Exclusion criteria: (1) suffering from severe acute or chronic diseases, including malignant tumours, chronic renal failure, cirrhosis, liver failure, chronic obstructive pulmonary disease and heart failure; (2) having severe motor dysfunction or mobility issues, such as long-term bedridden status, amputation, prosthetics or severe joint diseases; (3) poorly controlled medical conditions or refusal to participate; (4) suffering from severe cognitive impairment (such as Alzheimer's disease) or mental illnesses that compromise the ability to normally participate in the study or accurately complete questionnaires and cooperate with measurements; (5) taking medications that interfere with skeletal muscle evaluation, such as glucocorticoids; (6) refusal to undergo physical function assessments.

4.1.2 Diagnostic criteria for sarcopenia

Sarcopenia was diagnosed on the basis of criteria from the Asian Consensus Update on Sarcopenia Diagnosis and Treatment (2019) as follows: a decrease in muscle mass (a necessary condition) combined with a decrease in muscle strength or physical function. (1) Decreased muscle mass: SMI $< 7.0 \text{ kg/m}^2$ for men and SMI $< 5.7 \text{ kg/m}^2$ for women. (2) Decreased muscle strength: grip strength $< 28 \text{ kg}$ for men and grip strength $< 18 \text{ kg}$ for women. (3) Decreased physical function: 6-m walking speed $< 1.0 \text{ m/s}$.

4.2 Observed and Measured Parameters

Anthropometric parameters including gender, age, height, weight and BMI were collected, together with medical history.

The following body composition parameters were measured using an InBody 370S body composition analyser: SMI, BMI, soft lean mass, appendicular lean mass and percentage body fat.

Physical function assessment included handgrip strength and gait speed measurement.

Handgrip strength was measured using a Model WCS-100 electronic dynamometer (Xinman Medical). Participants gripped the device with maximum force using their dominant hand or both hands. Each test was repeated at least twice, and the maximum value was recorded.

For gait speed measurements, participants were instructed to walk through a 6-m testing area at their usual pace without acceleration or deceleration. The test was repeated at least twice, and the average value was calculated for analysis.

4.3 Screening of Serum Biomarkers for Sarcopenia

Differentially expressed proteins were detected using DIA quantitative proteomics technology (Tianjin Kingmed Medical Laboratory), with a cut off of $p < 0.05$ and fold-change ≤ 0.83 or fold-change ≥ 1.2 . Further analysis was conducted through Gene Ontology functional annotation, Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis, and protein–protein interaction network analysis. Candidate serum biomarkers were selected based on the results of these analyses, in conjunction with literature and biological function review. The serum levels of candidate biomarkers were measured using ELISA.

4.4 Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software. Normally distributed variables are expressed as mean $\pm$ standard deviation, while non-normally distributed variables are expressed as medians and interquartile ranges. Counting data are expressed as N (%). Intergroup comparisons were conducted using the independent sample t-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. Multiple groups were compared using one-way analysis of variance for normally distributed data and the Kruskal–Wallis H test for non-normally distributed data. Rate comparison between groups was achieved using the Chi-square test or Fisher’s exact probability method. Risk factors for sarcopenia were analysed using binary logistic regression analysis. Correlation analysis was conducted using Spearman’s method. Receiver operating characteristic curves were plotted to determine the best cut-off value for serum markers to diagnose sarcopenia on the basis of the maximum Youden index, as well as to evaluate their predictive value. $P < 0.05$ was considered indicative of statistically significant differences.

5. Conclusions350

In this study, we employed data-independent acquisition-based quantitative proteomics coupled with enzyme-linked immunosorbent assay validation to systematically screen and verify plasma diagnostic biomarkers for sarcopenia in community-dwelling older Chinese adults. Our results demonstrate that serum levels of FLNA and VCAN are significantly elevated in older adults with sarcopenia. FLNA was identified as an independent risk factor for sarcopenia and exhibited favorable diagnostic performance with an area under the receiver operating characteristic curve of 0.797, while VCAN showed moderate diagnostic value with high specificity.

These findings address a critical gap in primary care settings by providing novel serological candidate biomarkers that enable simple, feasible early screening for sarcopenia among community-dwelling older populations, for whom conventional diagnostic tools are often inaccessible. The identification of FLNA and VCAN also sheds new light on the molecular pathogenesis of sarcopenia related to cytoskeletal remodeling and extracellular matrix dysregulation, laying an experimental foundation for further mechanistic exploration and the development of targeted interventions. Future multicenter studies with larger, geographically diverse cohorts are warranted to confirm the generalizability and clinical utility of these biomarkers.

List of Abbreviations365

Abbreviation	Full Name
ACTB	β-actin
ALT	Alanine transaminase
AST	Aspartate transaminase
AWGS	Asian Working Group for Sarcopenia
AUC	Area under the curve
BMI	Body mass index
CC	Calf circumference
CI	Confidence interval
Cr	Serum creatinine
DIA	Data-independent acquisition
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
FLNA	Filamin A
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MEF2	Myocyte enhancer factor 2
NC	Non-sarcopenic control
OR	Odds ratio

ROC	Receiver operating characteristic	
SP	Sarcopenia	
SMI	Skeletal muscle mass index	
SPP1	Secreted phosphoprotein 1	
VCAN	Versican	
Declarations		366
Ethics approval and consent to participate		367
The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Affiliated Hospital of Chengde Medical University (protocol code CYFYLL2025572). All patients participating in the study signed the informed consent form.		368 369 370
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Competing interests		375
The authors declare that they have no competing interests.		376
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